LABORATORY AND FIELD EVALUATION OF THE MOLLUSCICIDAL PROPERTIES OF CALLIANDRA PORTORICENSIS (Jacq) BENTH (ULE)

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INTRODUCTION

Calliandra portoricensis (Jacq) Benth (Mimosaceae) locally known as ULE is a shrub which grows wildly in Nigeria. Although it has many medicinal uses it has never being implicated as a vegetable molluscicide. The aim of this report is to give details of the laboratory and preliminary field evaluation of the molluscicidal properties of the plant.

EXPERIMENTAL

Source of plant and extraction:

The plant material was identified by the staff of Forestry Research Herbarium, Ibadan, Nigeria to be that of Calliandra portoricensis. The root was collected from Ijare near Akure in May, 1979 The root bark was macerated in ethanol using waring blendor and left in ethanol overnight. On filteration, ethanol was removed in vacuo at 45°C Final traces of ethanol was removed by freeze-drying and the residues recovered as powder.

Molluscicidal test procedures:

Except otherwise stated, the WHO Method (3) for testing for molluscicides was followed,exposure and recovery period being 24 hours in all the rests. The number of replicates was 4 while the average number of snails per test was 10. Molluscicidal test results were analysed by probit analysis method (2) with 95% confidence limits where quoted.

Source of Snails:

Bulinus globosus were cultured in our laboratory with a slight modification of the method of McClelland as described by Adewunmi and Sofowora (1). Various age groups of the snails were used in some of the tests.

Effect on the development stage of S. haematobium:

Fifty ml of distil water containing 200 freshly hatched miracidia (from the eggs of S. haematobium) were mixed with 50 ml of double concentration of the freshly prepared ethanolic extractive of root in serial dilutions. During treatment periods of varying time, microscopical observations on the movement of the miracidia were recorded.

Effect on snail eggs, different age groups of snails and tests for possible degradation by physico-chemical factors:

These tests were carried out precisely as described by Adewunmi and Marquis (1980).

Toxicity to fish

Tilapia sp (40 in number and each 6-10cm in length) were exposed to serial dilutions of the ethanolic extractive for 24 hours. The fish and snail toxicity dexes were calculated as follows:

$$\text{Fish toxicity Index} = \frac{\text{LC50 of fish}}{\text{LC50 of snail}}$$

and

$$\text{Snail toxicity Index} = \frac{\text{LC50 of snail}}{\text{LC50 of fish}}$$

TABLE I

Molluscicidal effects of various concentrations of ULE on age group of Bulinus globosus.

Age of Snails	LC50 (ppm)
0-24 Hours (0.6 - 0.9 mm)	1.25
4 weeks old (3.8 - 5.0 mm)	5.0
9 weeks old (8.8 - 10.00 mm)	5.5

Preliminary field evaluation.

Measured doses of the ethanolic extractive of the plant were dissolved in water after approximating the volume of water of a stagnant pond at Fasina near Ile-Ife, Nigeria. The measured toxicant was then sprayed into the pond. The pond which is about 40 sq. meters was surveyed for estimating the snail population before applying the toxicant. This was carried out by snail scoops every 5 meters. The snail number per 100 scoops was calculated. Snail number was determined twice every week for three weeks before treatment and four weeks after treatment. Another pond close by was used as control for comparison.

RESULTS

High concentrations (100–500 ppm) of the ethanolic extractive was toxic to the miracidia of Schistosoma haematobium while laboratory molluscicidal concentration had no effect. No ovicidal activity was shown even at a concentration of 500ppm over a period of 10 days to exposure. The susceptibility of the various age groups to our candidate molluscicide is shown in Table I. The results showed that the newly hatched snails were more susceptible to the toxicant while there was little difference in the susceptibility of the mature and 4 week old snails. Table IV shows that the fish tested was about 28 times more susceptible to the toxicant than the snails.

TABLE II

Comparison of Molluscicidal activities of irradiated and untreated control dillution of ULE and Bayluscide.

	LC50 (95% confidence limits) in ppm	
Molluscicide	Irradiate	Untreated
ULE	7.49 (5.01-9.11)	5.62 (4.93-6.64)
Bayluscide	0.20 (0.17-0.32)	0.09 (0.06-0.10)

*Exposed to a U.V. lamp (Gallenkamp LH 530) with a peak at 366 mm for 8 hours.

TABLE III

Effects of Physio-chemical factors on the molluscicidal activity of Ule

TEST	LC50 (95% confidence limits) in ppm
1. Effects of mineral	7.49 (6.55 - 8.49)
Control (untreated group)	5.55 (4.60 - 6.80)
2. Inactivating effect of organic matter	5.21 (4.9 - 6.0)
Control (untreated group)	5.25 (3.95 - 5.8)
3. Inactivating effect of hydrogen ions	
PH 4.2 - 4.9	5.01 (4.3 - 6.95)
PH 6.8 - 9.0	5.01 (4.42 - 6.88)
Control (untreated group)	5.35 (4.2 - 7.1)

The extract however appears relatively stable under various physicochemical factors (Tables II and III) except under the influence of ultraviolet light where the activity is slightly decreased but the decrease is not as much as bayluscide, the activity of which was reduced by about 2.2 fold.

In the preliminary field evaluation, Bulinus clobosus was reduced from the pretreatment density of 180 snails per 100 scoops to about no surviving snail/100 scoops in the first, second, third and fourth week post treatment. Lanistes sp was reduced from 2,009/100 scoops to about 1,000 in the first week, 500/100 scoops in the second week, 100/100 scoops in the third week and 20/100 scoops in the fourth week post treatment. Young Tilapia sp were killed while the adult were not appreciably affected. There was no apparent effect on aquatic plants such as Pistia sp and Nymphia lotus.

TABLE IV

Toxicity of ule ethanolic extractive on Bulinus globosus compared with its toxicity on Tilapia sp.

	LC50 with 95% confidence limit	Toxicity Index
Snail	5.5 (4.52 – 6.7)	27.5
Fish	0.20 (0.10 – 0.52)	

DISCUSSION

The results show that the ethanolic extractive of the root bark is stable under various environmental factors such as pH (4.2–9.0), ultraviolet light and presence of organic matter. The shortcomings of this molluscicide include very low fish toxicity index and its non ovicidal activity; but its non ovicidal activity can be overcome by multiple tratments timed to kill the adult and juvenile snails.

The LC50 of Aridan (Tetrapleura tetraptera) against Bulinus globosus was found by the authors to be 2.01 (1.94-2.65)ppm (3). The LC50 of the ethanolic extractive of Ule was found here to be 5.25-5.62ppm while the one for Jatropha gossypiifolia was 11.55-16.24ppm (2). It is interesting to note from these earlier investigations in our laboratory that Ule is about 2.2 times less as potent as Aridan which is currently undergoing field trials in the surrounding villages.

The preliminary field evaluation gave encouraging results at a concentration of 20ppm confirming our laboratory findings. From the results obtained if more snail habitants are treated all over the country, the snail population will definitely drop to a level that might break the schistosome infection cycle.

ACKNOWLEDGEMENTS

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AN EVALUATION OF SOME COMMERCIAL BRANDS OF AMPICILIN CAPSULES AVAILABLE IN NIGERIA

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SUMMARY

Ten commercial brands of ampicillin capsules available locally were subjected to weight variation, uniformity of content, disintergration time and dissolution rate tests. The methods used were those recommended in the United States Pharmacopoiea (U.S.P) and the British Phrmacopoiea (B.P) with slight modifications in some tests.

Considerable variations in these parameters were observed from brand to brand. Some brands failed some of the tests and passed others. A particular, brand was found to have an average ampicillin content of only 73.6% while, the 90% dissolution of the active drug from another brand was not achieved even after the end of an hour. However, a few of the brands passed in all the tests.

Statistically significant correlation was found between weight variation and content uniformity results. Also, a good rank order correlation between disintegration time in acid medium and time for 50% dissolution (i.e. T50%) was obtained.

No significant difference was observed between the dissolution rates of capsules containing the anhydrous ampicillin and those containing ampicillin tri-hydrate.

The results emphasizes the need for a stringent quality control on ampicillin capusules being imported into the country before they are made available to the Nigerian market.

INTRODUCTION

Capsules are among the most common oral unit dosage forms currently in use, being next only to tablets. Although their formulation requirements are minimal when compared to tablets, various studies have indicated that the ultimate properties of capsules are profoundly influenced by the ingredients and procedure employed in the formulations and the manufacturing process (1, 2, 3, 4, 5). Such formulation effects are manifested in-vivo as differences in physiological availabilities and therapeutic efficacies of generic brands of a given drug and this has been aptly demonstrated by various workers. In a review of these studies, Florence (6) pointed out that the use of in-vitro dissolution rate and disintegration time studies to predict such in-vivo behaviour of solid dosage foms is restricted by the frequent lack of correlation between in-vitro and in-vivo results. Nevertheless, an indication of such in-vivo behaviour can be obtained from in-vitro tets(7). In addition, they are less expensive and more easily performed than the in-vivo tests hence, they are employed for the routine quality control of capsule formulations, in conjunction with other tests, such as weight variation and content uniformity.

Ampicillin is an antibiotic powder used for the general treatment of microbial infections. Although, it is available as powder (which requires reconstitution with water into a suspension before administration) and as injections, the main form in which it is presented is as capsules. Its potential for formulation problems is as great as that for any other drug in capsule form, and this, coupled with its popularity as shown by various studies (8,9) have prompted some drug regulatory bodies to request that dissolution rate tests be performed as a routine quality control procedure on ampicillin capsules (10). In addition, various studies have produced conflicting results as to the differences in dissolution rates between commercial ampicillin capsules containing the anhydrous ampicillin and those containing the less soluble trihydrate.

Most drugs consumed in Nigeria today, including ampicillin capsules, are imported and the few studies carried out on some of these have indicated that many are of low standard in terms of quality and efficacy (11, 12). However, none of these studies have been carried out on ampicillin capsules. Besides, we have observed that of the numerous genric brands of ampicillin capsules available, any brand is dispensed in our hospital pharmacies for such reason as costs and shortage of drugs without any knowledge of the quality of these brands. It is for these reasons that the present study was undertaken to investigate the variations in average weight, uniformity of content, disintegration time and most importantly, dissolution rates among ten commercial brands of ampicillin capsules. An attempt was also made to show the differences, if any, between capsules containing anhydrous ampicillin and those containing the trihydrate form. The effects of temperature on the dissolution rate of some of the brands were also studied.

MATERIALS AND METHODS

Materials:

Ten different brands of ampicillin capsules, each labelled as containing the equivalent of 250mg. of ampicillin were purchased from local retail pharmacy shops. Their relevant informations and designations are shown in Table I.

Experimental Methods:

Weight Variation – The U.S.P. (13) method was used. Twenty capsules from each brand were weighed individually and the mean weight was determined. The standard deviation (6), and coefficient of variation (C.V.) of the weight were calculated.

TABLE 1

List of Commercial brands of Ampicillin capsules used in this study

Code	Form of Ampicillin	Country of Origin	Batch Number	Expiry Date
A	Trihydrate	India	ROC/-33-77	8/79
B	Trihydrate	Italy	1020	2/80
C	Trihydrate	W.Germany	40797/72315	6/81
D	Anhydrous	Yogoslavia	772445	4/80
E	Trihydrate	Italy	1264/1578/A	4/79
F	Trihydrate	W.Germany	196	9/80
G	Anhydrous	Italy	237	2/80
H	Anhydrous	Nigeria	22777	7/80
I	Anhydrous	U.K.	9972	2/80
J	Anhydrous	U.K.	1586	2/80

Note: All the capsules were inside their expiry date when this study was undertaken.

TABLE 2(a)

Results of the weight variations for the different brands of ampicillin capsules

Brand Code	Mean Weight (mg)	Weight Range as % of of mean weight	Standard deviation (mg)	Coefficient of variation (%)	Compliance with U.S.P.
A	410.3	93.55-102.85	10.73	2.62	Passed
B	394.2	84.68-109.66	25.59	6.49	Failed
C	381.1	96.12-105-04	8.99	2.36	Passed
D	392.9	62.71-109.14	37.61	9.57	Failed
E	377.7	90.23-107.55	17.82	4.72	Passed
F	447.2	96.13-102.01	7.22	1.62	Passed
G	367.1	97.98-102.04	4.59	1.25	Passed
H	359.7	91.58-103.64	10.48	2.91	Passed
I	375.6	91.69-107.51	17.44	4.64	Passed
J	371.9	96.86-102.75	5.50	1.48	Passed

TABLE 2(b)

Results of further weight variation tests on brands B and D

Brand	B	D
Mean weight of Capsule content (mg)	317.4	309.0
Range as % of mean net content	81.41-110.02	53.14-112.07
No. of capsules beyond the limits 90% — 110%	2	3
No. of Capsules beyond the limits 75% — 125%		1
Compliance with U.S.P. specification	Passed	Failed
Capsule Shell Weights		
Standard deviation (mg)	1.93	1.92
Coefficient of variation (%)	2.45	2.43

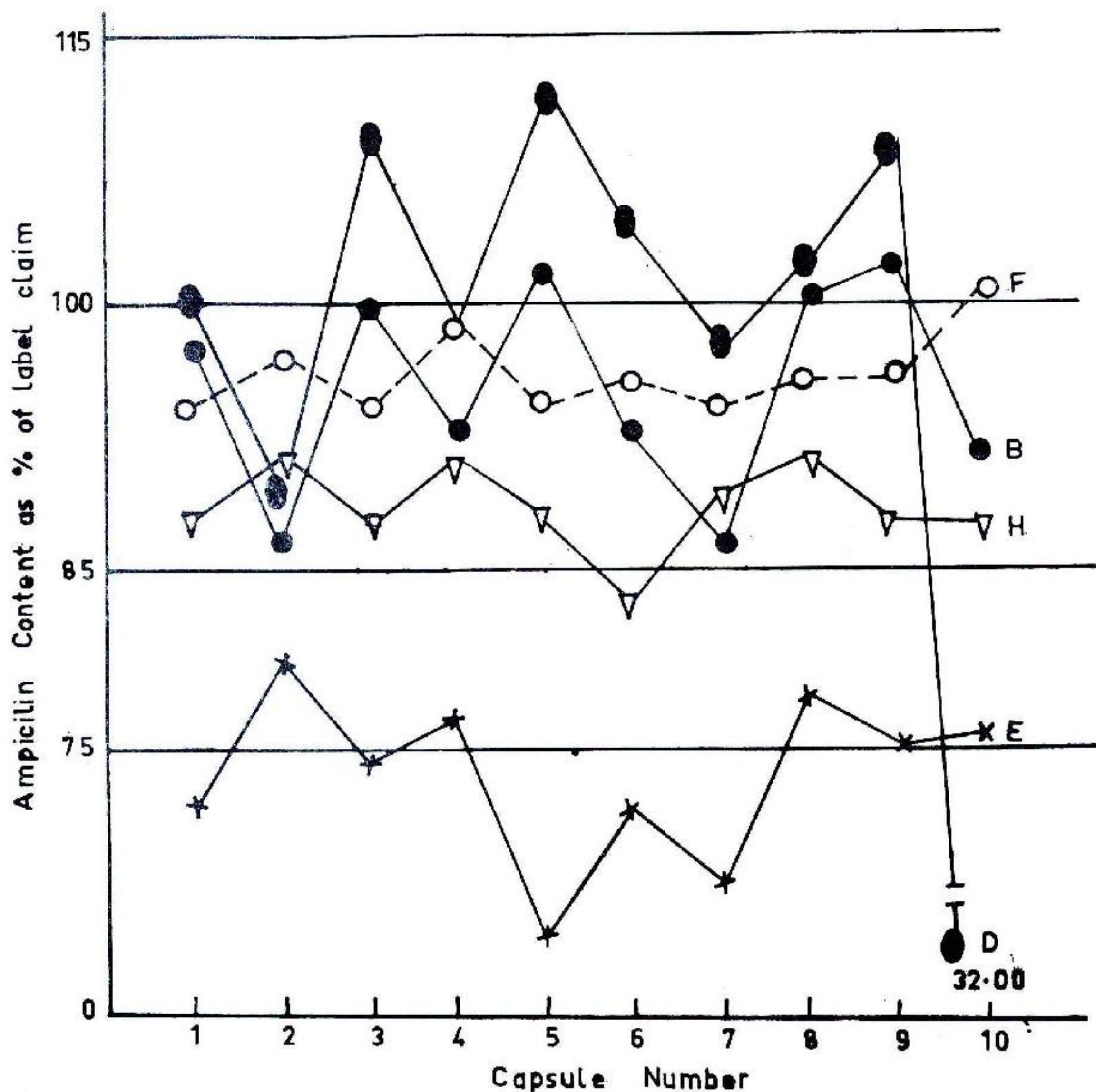


FIG. 1A. Variation in Uniformity of Contents of ampicilin per Capsule, Brands F, B, H, E, D

When the weight of any of the capsules fell outside the limits, 90-110% of the mean weight, each capsule was then emptied, the weight of its contents obtained and the mean calculated.

Content Uniformity:

Ten capsules were randomly selected from each batch and the contents of each capsule was determined by the B.P. (14) assay method for ampicillin capsules at an absorbance of 322 nm with a Unican SP 500 series 2 Spectrophotometer, and by referring to a calibration graph obtained from a solution of ampicillin trihydrate powder (Beecham Research International–Apapa).

Disintegration Time:

B.P. Disintegration method – The test was carried out in a Manesty Disintegration Tester using the B.P. method. Distilled water was used as the disintegrating fluid and the temperature was maintained at 37± 0.5°C. Five capsules were placed in each basket, the determination was done in triplicate and averaged.

The test was then repeated by placing only one capsule in each basket and noting the longest time for one of the capsules to completely disintegrate

Modified U.S.P. Disintegration Methods:

The procedure was the same as above but only one capsule was placed in each basket and the disintegration time was taken as the mean of ten runs.

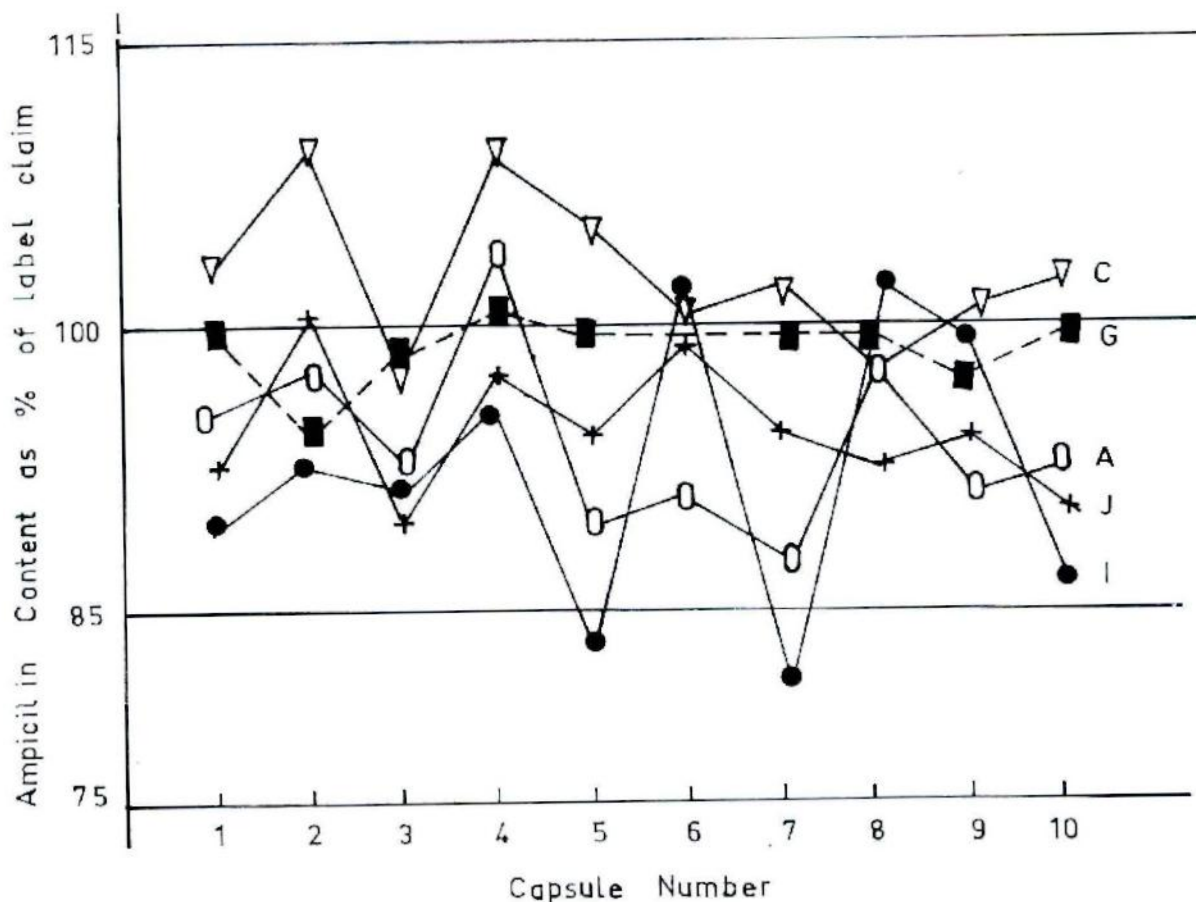
Distintegration in Simulated Gastric Fluid:

The test was repeated for individual capsules using instead of water, simulated gastric fluid T.S. (without pepsin) U.S.P. The mean was calculated from six runs.

Dissolution Rate:

A modified form of the U.S.P. dissolution rate apparatus was used. It consisted of a 1 litre beaker containing 900ml. of simulated gastric fluid T.S. (without pepsin) U.S.P., a doubly-wound basket made from a No. 16 mesh size wire gauze, attached to a rod connected to a variable speed motor and rotated at 120 r.p.m. The beaker was immersed in a water bath maintained at 37 + 0.5oC (unless otherwise stated).

FIG. 1B. Variation in Uniformity of Content of Ampicil in per Capsule A, J, I, G, C.



1ml. samples were withdrawn at different time intervals and rapidly filtered. The concentration of ampicillin that had dissolved in the medium during the period concerned was determined in the same way as for the content uniformity test. The test was performed in triplicate for each brand except in the case of brand D, where five individual capsules weretested to illustrate the intra-batch variation.

The effect of temperature was determined by repeating the test on the slowest dissolving brand over a temperature range of 31°C to 44°C.

RESULTS AND DISCUSSION

The U.S.P. specification on weight variation requires that in the initial tests, none of the twenty capsules should deviate in weight from the mean weight by more than 10%. This requirement was fulfilled by all the brands except B and D as shown in Table 2(a). In order to ascertain whether the observed deviation for these two brands was due to variation in capsule

shell weight or not, a further test was performed. The results, Table 2(b) show that the variation in both cases was not due to variation in shell weight, since the shell weights had standard deviations of 1.93mg. and 1.92mg. and coefficients of variation of 2.43% and 2.45% respectively. The U.S.P. specification which states that the weights of the contents should not be more than 10% in any two capsules and in no capsule by more than 25%, was met by brand B but, not by brand D. On opening the brand D capsules, it was observed that there was considerable variation in depth in fill. This seems to imply that the U.S.P. test, may in fact have failed the B.P. test if it had been applied to them.

Applying the standards reported by Pietra and Setnikar (16) for tablets in which a coefficient of variation not exceeding 2% indicates excellent uniformity of weight, it can be seen from Table 2(a) that brands F, G and J which showed very little variation are excellent while, brands A, C and H are only just good. On the contrary, brands I, E, B and D exhibited marked variation (the variation increasing in that order) and this is reflected in the fact that brands B and D failed the initial weight variation tests.

The results on the content unformity, Figs. 1(a) and (b), show marked variations similar to that of the weight variation test.

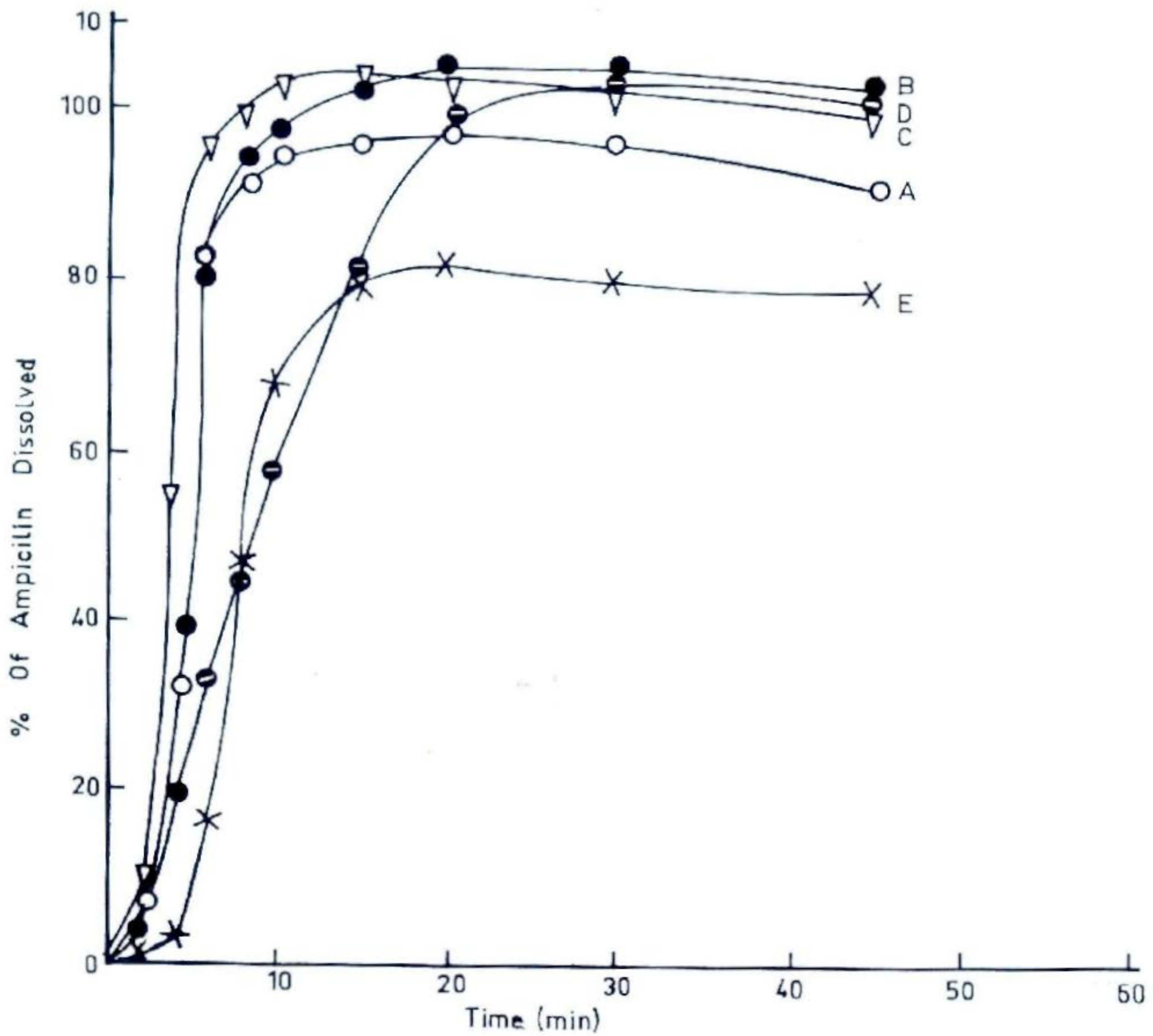


FIG. 2A. Dissolution Profiles of Brands A, B, C, D. and E at 30%.

TABLE 3

Results of disintegration time tests

Brand Code	D.T. for 5 capsules in one basket (min)	Longest time for one capsule (min)	Mean D.T. for 10 capsules (one per basket (min)	Range of D*.T. for the 10 capsule (min)	D*.T. in simulated Gastric Fluid (min)	Remarks
A	9.5	6.0	5.5	4.4-7.1	5.4	Passed
B	21.1	8.2	8.2	7.0-10-08	6.4	Passed
C	18.4	13.5	8.6	6.2-14.4	4.6	Passed
D	31.5	16.2	15.5	13.0-21.4	11.5	Failed+
E	17.2	11.3	10.1	8.4-11.5	6.2	Passed
F	19.2	17.6	15.4	7.6-20.3	13.5	Failed+
G	8.2	6.1	5.4	5.5-6.5	5.3	Passed
H	16.5	11.4	10.4	8.5-13.1	6.5	Passed
I	27.5	27.2	14.2	6.6-27.3	7.1	Failed+
J	11.1	6.5	6.1	5.0-7.3	5.2	Passed

*: D.T. = Disintegration Time
+: Failed B.P. specifications only.

TABLE 4

Summary of all results

Brand Code	Weight	Content Uniformity		Disintegration Time (Min)		Dissolution RAte		Overall Ranking
	Coefficient of variation (%)	Mean Content (%)	Coefficient of variation (%)	B.P. Individual Capsule Test	U.S.P. (Mean)	T50% (Min)	T90% (Min)	
A	2.62	93.93	4.79	6.0	5.5	4.3	6.0	3
B	6.49	95.20	6.24	8.2	8.2	4.5	6.6	6
C	2.36	102.69	3.76	13.5	8.6	3.6	4.4	4
D	9.57	95.44	23.10	16.2	15.5	8.8	16.5	10
E	4.72	73.55	6.23	11.3	10.1	8.2	+	7
F	1.62	94.49	3.63	17.6	15.4	25.8	60	8
G	1.25	98.49	1.62	6.1	5.4	3.8	6.4	1
H	2.91	88.49	2.58	11.4	10.4	5.0	12.6	5
I	4.64	92.32	7.69	27.3	14.2	6.8	10.0	9
J	1.48	96.17	2.21	6.4	6.1	4.3	6.0	2

+ Brand E released only 83% of ampicillin.

It is evident from the results that brands A, B, C, F, G and J all passed the test with no capsule outside the 85%-115% limits as specified in the U.S.P. Whereas brand H only just passed as one of the capsule had ampicillin content below the 85% limit but above the 75% limit. The result also shows that two capsules of brand I were below the 85% limit and therefore this brand failed the test. Similarly, brand D and E failed because the ampicillin content of a capsule of brand D was 31.95% while no capsule of brand E had ampicillin content of up to 85%. In fact, the mean content for brand E was 73.55%, and since all the capsules were found to be fully filled on opening (as reflected by the low variation of the capsule weight--it had a coefficient of variation of 4.72%), the low ampicillin content could be attributed either to underweighing of the ampicillin during production of the batch, or to the use of an old stock of ampicillin in the manufacture. The fact that the expiry date for this brand was only one month away from the time of the assay may be responsible for this low ampcillin content, because if an old stock of ampicillin had actually been used, its potency would have reduced considerably before the labelled expiry date. The general practice is to give an allowance of about three years for expiration (from the time of manufacture) within which period the potency of the drug substance should not fall below 90% of its original, labelled value (17). Another reason may be due to poor storage conditions of this brand since the time of its manufacture which may have led to some degradation of the ampicillin.

Calculations of the standard deviation (6) and coefficients of variation (C.V) show that brand G had the lowest variation (6-4mg.) while, brand D had the highest (6-55.13mg).

Although no official limits have been set for these parameters, they give a clear indication of the real objective of the content uniformity test (i.e. variation from capsule to capsule). Statistical analysis of the coefficients of variation obtained in this test and those in the weight variation test employing the student's 't' test, shows that there was significant correlation at the 95% confidence level (P 0.05) and also, it seems to justify the fact that the U.S.P. specifies limits for content uniformity only as an alternative to weight variation, since the order of variation in both tests were approximately the same. However, the results of brand E which had an "excellent" weight variation but a "poor" ampicillin content, illustrates the necessity of both tests in order to fully characterise a product.

Data on the disintegration tests of the different brands of ampicillin capsules are summarised in Table 3. Determination of the end point of the disintegration time proved difficult when testing five capsules in one basket since the capsules softened and formed an adhesive gelatin mass which stuck to the mesh and entrapped within it, some of the solid content. Thus, as can be seen from the date, only brands A, G and J passed the five-capsule-per-basket test. Brands C, E and H failed due to aggregation of the ampicillin capsules which was clearly evident during the test. However, these three brands passed the repeat test using only one capsule per basket. Similarly, brand B failed the five capsule/basket test due to the presence of some large granules but, eventually passed the repeat test of one capsule/basket. Brands I and F however failed because of the presence of large and poorly water permeable aggregates which remained in the disintegration medium after the gelatin shell had completely dissolved. They also failed the repeat tests due to the formation of these water impermeable aggregates which was observed to occur mainly around the cap-body junction, indicating a low porosity of these brands. It can be inferred that their poor porosity may have resulted from the application of excessive "pressures" during capping of the capsule body, which led to the compression of some of the powders around the junction. In addition, the high proportion of fines in the solid content of both brands may also have contributed to the observed found to exhibit poor porosity after compression(4).

Brand D exhibited a peculiar disintegration in that one half of the capsule shell was hardly water permeable with the result that it never dissolved or disintegrated in the course of any of the tests. Because some solid content were stuck to this part of the capsule shell for a long time, they could not be released within the specified limit, and as a result, the brand failed.

It is evident, Table 3, that in general, the disintegration times for the repeat test (i.e. one capsule/basket) were shorter than those for the five capsule/basket test due to the aggregation which occurred with the five capsules tested together.

A similar observation made by Jones and Cole (3), led them to suggest an alternative in the B.P. specifications such that only one capsule/basket should be used instead of five and the disintegration time being calculated as the mean of five or more determinations as is the case in the U.S.P.

U.S.P. specifies that capsules shoud disintegrate between 15 and 30 min. This relatively liberal limit made it possible for all the brands to pass the U.S.P. test as shown in Table 3. Nevertheless, brands C, D, F and I exhibited wide ranges in disintegration times the difference between the fastest and slowest being not less than 8 min. in all the four brands.

The results, Table 3, also shows that all the brands showed faster disintegration in simulated gastric fluid than in water, largely because the gelatin shell was more soluble in the simulated gastric fluid (3). However, brand D still exhibited its characteristic of one half of the capsule failing to disintegrate, although this insoluble part finally broke up into shreds within 12 min.

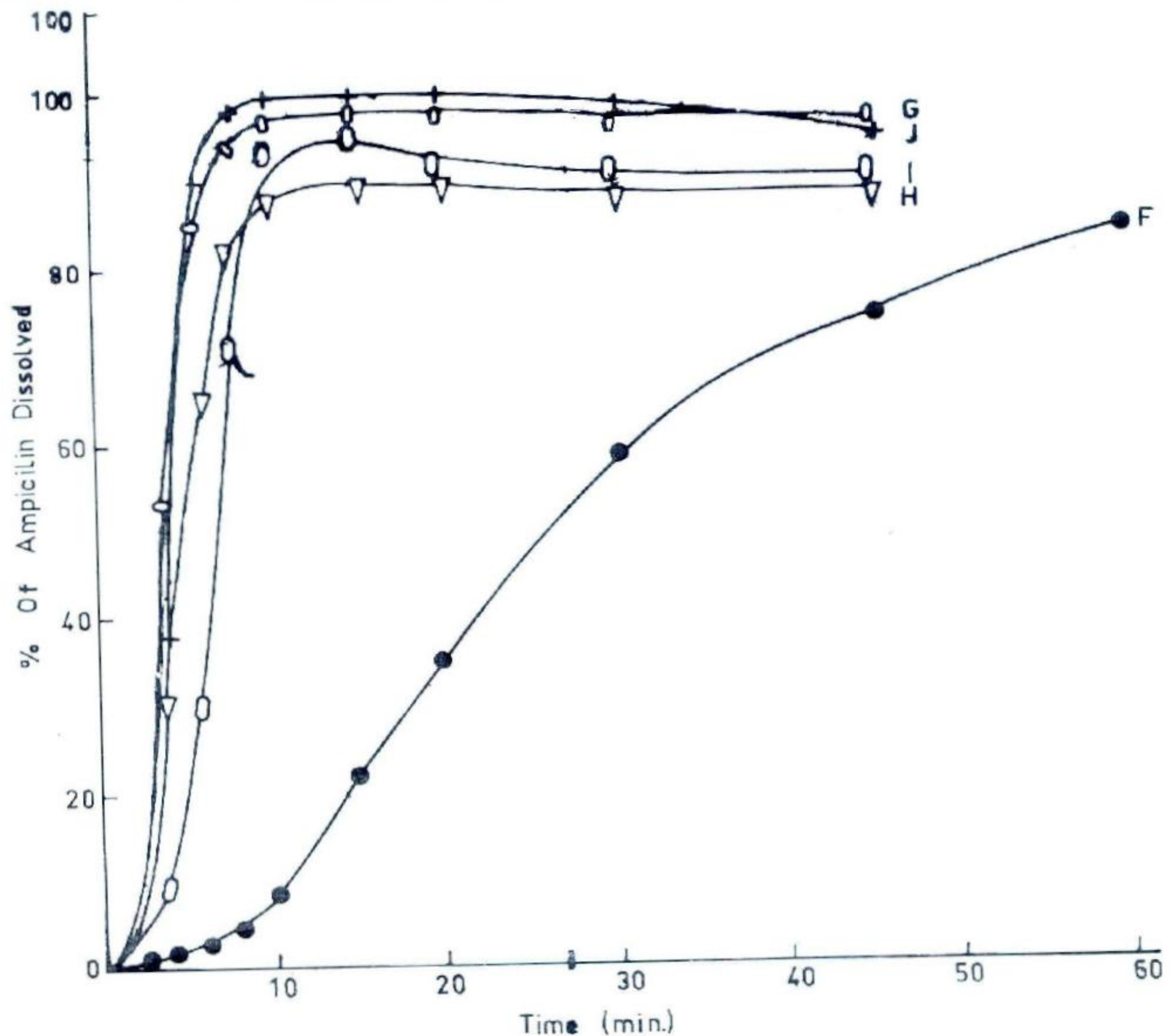


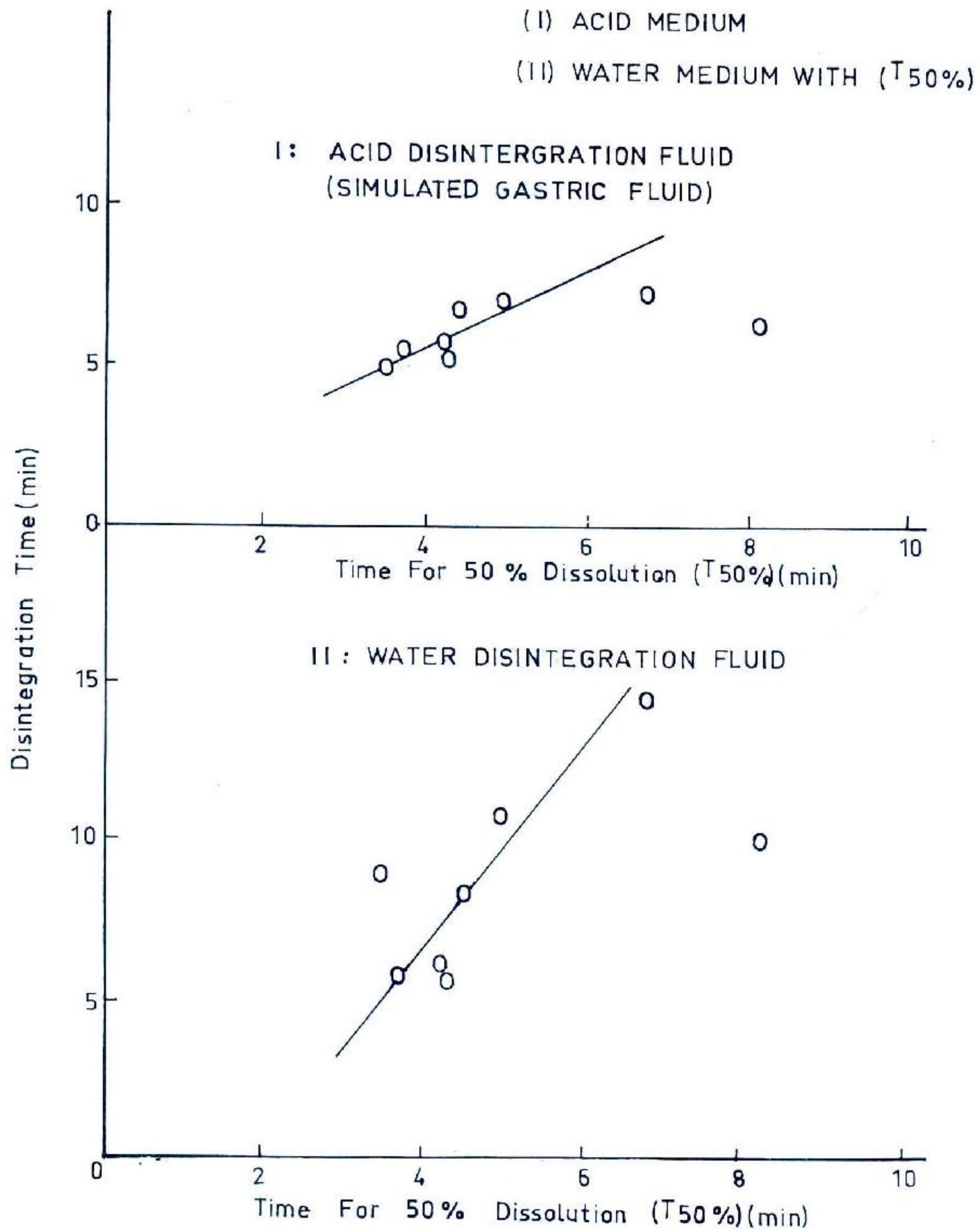
FIG. 2b Dissolution Profile of Brands F,G,H,I and J at 37°C

The results of the dissolution rate tests are plotted in Figs 2(a) and (b). Due to the absence of specifications in both the B.P. and the U.S.P. on dissolution rate of ampicillin capsules the test is at present essentially a comparative one. In fact, a recent B.P. policy statement on dissolution testing of ampicillin capsules (10) merely regards the test as a measure of proportion of drug capable of going into solution under standardised in-vitro testing conditions within a reasonable time. What this "reasonable time" should be, is currently under investigation. Nevertheless, from Figs. 2(a) and (b) it can be seen that brands C. G. J. A, H and I exhibited good dissolution rate profits since their T50% ranged between 3.3 and 6.5 min., while brands D and E have slower dissolution rates with T50% of 8.5 and 8.1 min. respectively. For brand D, the gelatin cap did not dissolve as it did in the disintegration test, hence adherent ampicillin particles were only slowly released from it and a slow dissolution rate was observed. Although brand E exhibited a good disintegration time, the maximum percentage of ampicillin dissolved was only 81.6%. This is in good agreement with the low mean content of ampicillin recorded for this brand (see Fig. 1(a). Consequently, the slow dissolution rate is more likely due to this low mean content of ampicillin since dissolu-

tion rate follows roughly a first order rate law. This is supported by the fact that brand C which had the highest mean content of ampicillin (see Fig. 1(b) also had the shortest T50% of 3.6 min.

Brand F had the poorest dissolution rate profile of all the brands studied and it was observed that once the gelatin shell dissolved, the solid content formed a lumpy mass or aggregate which settled to the bottom of the basket. The release rate from this aggregate was so slow that even at 45 min. only 75.7% of ampicillin was in solution. The inability to deaggregate, coupled with the equally poor disintegra-

FIG. 3. CORRELATION OF DISINTEGRATION TIMES IN:
(I) ACID MEDIUM
(II) WATER MEDIUM WITH (T50%)



tion of this brand, shows that the presence of the labelled amount of active ingredient in capsule formulations does not necessarily imply that the active ingredient would be made available to the body on ingestion of the capsule.

Fig 3 shows the correlation between the disintegration times and the time required for 50% dissolution of ampicillin from the capusles. Although the correlation was not very significant, the results showed a good correlation when ranked (coefficient of rank correlation was found to be 0.842) and this implied that the differences in the disintegration times and T50% were of the same order in all the brands. It also seems to justify the earlier reliance of most official compendia on disintegration time tests as an indicator of release rate.

Comparison of the dissolution behaviour of brands containing the trihydrate and those containing the anhydrous forms of ampicillin, by means of the student's 't' test (18) showed the difference in mean value of T50% for the two forms to be statistically insignificant (p 0.1). This suggests that the dissolution of both forms of ampicillin is essentially similar and thatthe variations in dissolution rates observed in the present study on the different brands must be due to differences in their formulations and manufacturing processes. This is in agreement with the results obtained by Baner (19), Whyatt at al (20) and Hill et al.

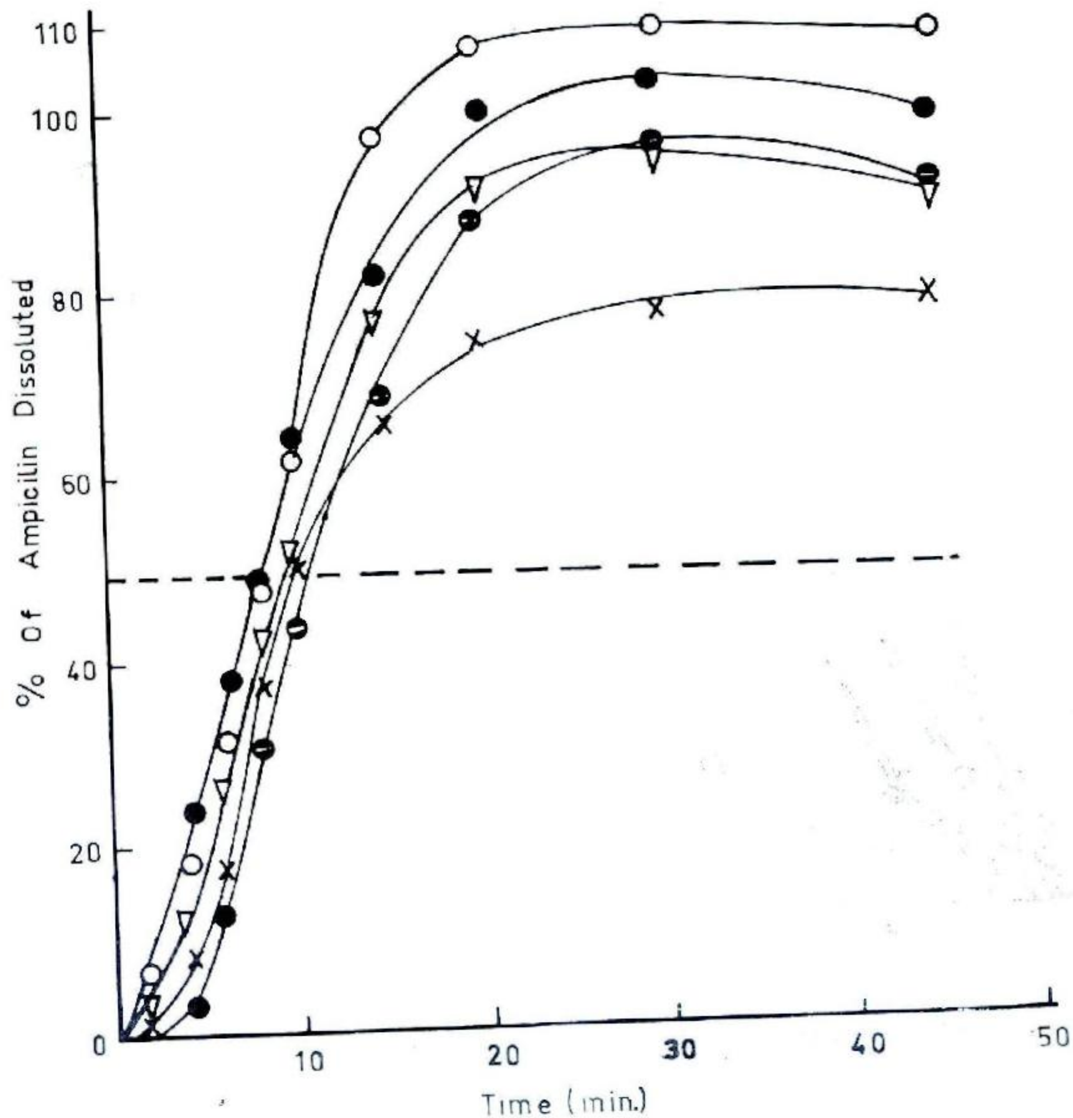


FIG.4. Intra-batch Variction in dissolution rate profile within Brand D.

(21) who showed that formulation factors exert a greater effect on the dissolution rates of ampicillin capsule formulations than the absolute solubility of the ampicillin powder..

Brand D was chosen to illustrate the variation in dissolution rate profiles within a batch because it exhibited the greatest variation in content uniformity. As shown in Fig. 4, the dissolution behaviour reflects this variation in ampicillin content. The values of T50% ranged from 7 to 11 min. whereas, the T90% ranged from 14 to 23 min. for four of the capsules; The fifth capsule, released a maximum of only 80% of ampicillin even after an hour. This variation would result in fluctuating blood levels when this brand is administered orally.

Fig. 5 illustrates the effect of temperature on the dissolution rate of brand F and it is clearly evident from the plots that a change of temperature from 31.50C to 44oC increased the dissolution rate by as much as 600%.

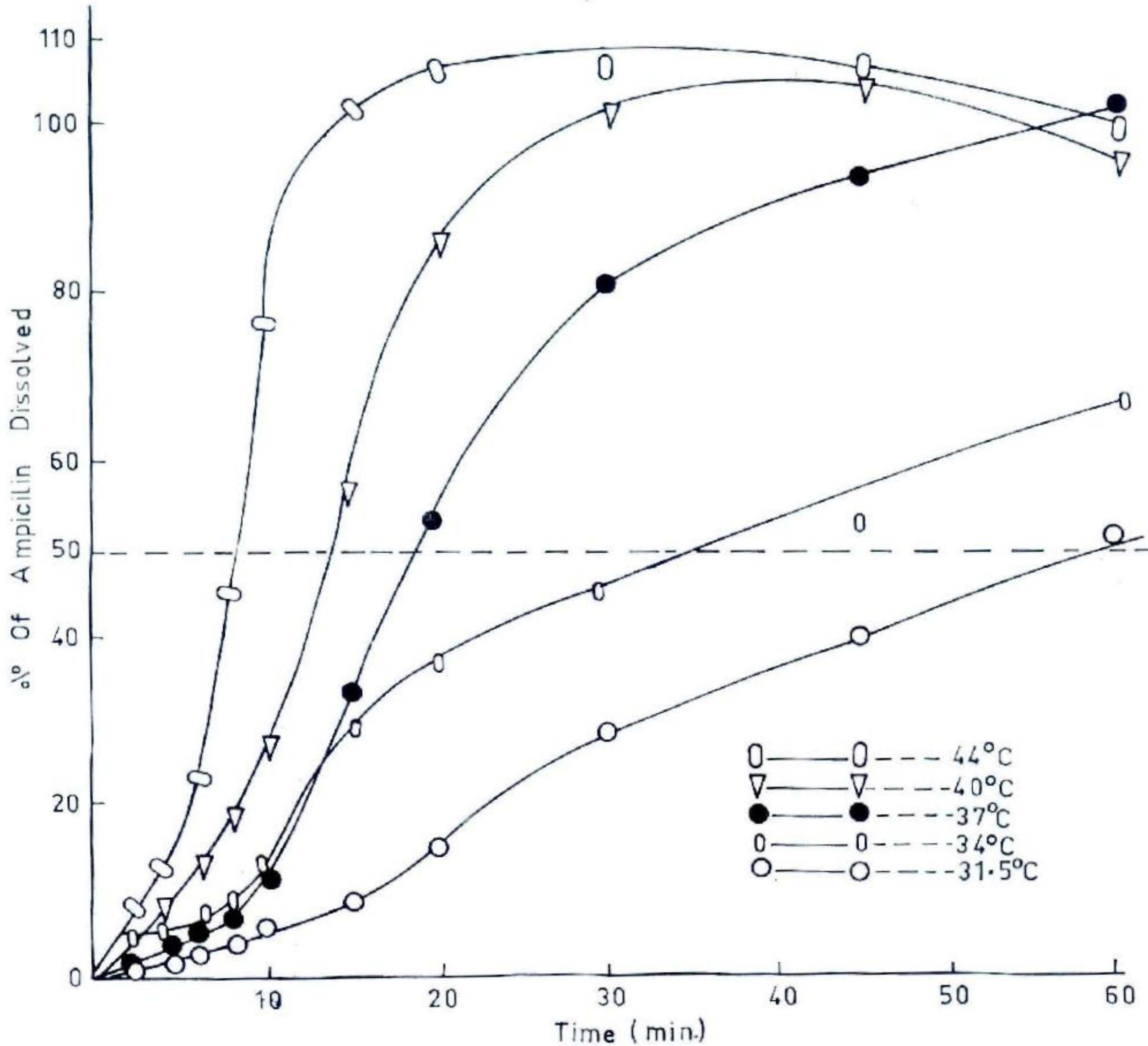


FIG. 5: Effect of Temperature on Dissolution Rate profile of Brand. F

Finally, Table 4 shows a summary of the results of all the tests performed. Assuming all the tests to be of equal importance, an attempt was made to rank the ten brands on the basis of overall performance in all the tests and the following rank order was arrived at G J A C H B E F I D (i.e. Brand G is the best of the

brands, while brand D had the poorest performance).

CONCLUSIONS:

The results of weight variation tests can reasonably indicate the uniformity of content of a hard gelatin capsule, as shown by the correlation between the two. This, however, is not always so, since, in the case of brand B, a good weight variation did not correspond with the content of ampicillin.

Disintegration tests carried out on five or more capsules singly, and, calculating the mean of the results, are better at portraying the disintegration behaviour of a capsule formulation, since agglomeration occurs when multiple capsules are used in one basket. In addition, the test on individual capsules correlates more with dissolution rate tests than those from multiple capsule tests.

It has been found that the disintegration of ampicillin capsules in acid medium is shorter than in distilled water medium and its dissolution rate is faster at higher temperatures.

A good content uniformity result is not enough to characterise fully the in-vitro release patterns of a capsule formulation since, if it fails to disintegrate and dissolve, to release the active ingredient, its desired therapeutic effects will not only attained. This was demonstrated by brand F.

The poor results obtained for content uniformity and dissolution rates for some of the brands (particularly bands B, D, E, F and I) emphasises the importance of adequate quality control of all pharmaceutical products imported into the country. This will eliminate bad products and prevent the undermedication of a patient when a generic brands of ampicillin formulation is administered.

ACKNOWLEDGEMENTS

We wish to thank Mr. J.G. Key of Beecham Research International, Apapa--Lagos for the gift of the pure ampicillin trihydrate powder and the Chief Pharmacist of U.B.T.H., Benin City for providing two of the brands.

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Silicon Derivatives of Medicinal Agents: Derivatives of P-Aminosalicylic, Salicylic and Benzoic Acids

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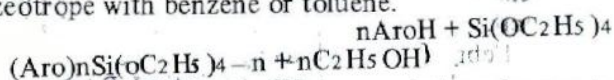
SUMMARY

Synthesis and characterisation of organosilicon derivatives of P-Aminosalicylic, Salicylic and benzoic acids are described. P-Amino Salicylic, Salicylic and benzoic acids are medicinal compounds which have been used as antitubercular, antibacterial, antifungal, Keratolytic (dermatophytoses) agents and also as preservatives. Silylation of these compounds with organosilicon reagents in acetone, tetraethoxy-silane, toluene and benzene have been prepared. These new silicon derivatives of benzoic, salicylic and P-aminosalicylic acids were obtained (i) by refluxing P.A.S. with silicon tetrachloride in tetraethoxy-silane in a molar ratio (4:1) to give compound (1) as hydrochloride. Similar compound was obtained in good yield when the same reaction (i) was carried out in acetone by stirring at 25°C) The reaction of P.A.S. with tetraethoxy-silane in molar ratios (1:2) or (1:4) carried out in benzene or tetrahydrofuran (ii) gives an unexpected interesting identical complex compound (ii) with a high quantitative yields in each of the two solvents.

Tetraethoxy silane is the reaction medium of salicylic acid with silicon tetrachloride in an equimolar ratio (1:1) to give compound (iii). This reaction presumbaly undergoes two stage reactions. The first state reaction presumably gives benzo-2,2-dichloro-2-sila-1, 3-dioxan-4-one which later reacted with tetraethoxy-silane to give benzo-2,2-diethoxy-2-sila-1,3-dioxan-4-one(iii).

The ethoxysilyl derivatives of salicylic and benzoic acids (iv) were synthesized by refluxing the acids with an excess of tetraethoxy-silane in benzene or toluene and the fractional distilation of ethanol formed as an azeotrope with benzene or toluene.



$$nAroH + Si(OC_2H_5)_4 \rightarrow (Aro)_nSi(oC_2H_5)_{4-n} + nC_2H_5OH$$

The identities of these compounds have been established by their elemental anlyses, infra-red spectra, melting points and Mass spectral analyses.

The derivatives are of interest, since silylation of medicinal agents has been known to alter their physico-chemical properties and Pharmacokinetics[1]. The purpose of this work was not only to establish conditions at which the silyl derivatives of these medicinal active acids could be synthesized but also to examine their biological activities and their release rates from an oitment base which are going to be reported later.

However, since P.A.S. is one of the antitubercular agents, the presence of silicon in the P.A.S.-silicon complex may presumably further prolong and increase the antitubercular activity of P.A.S. in the body. Reports showed that silicon is present in nearly all the body tissues and its compounds are less toxic when applied orally. The activity of each of the antitubercular compounds is suppressed or inhibited by the addition of certain metal ions e.g. Cu^{2+}, Co^{2+}, Ni^{2+}, Ca^{2+} and mg^{2+} has been reported.[2,3] Of these metals or metal ions, silicon does not possess such an inhibitory effect.

Since Phenyl-P-amino salicylate is an oily ester and was reported to have been administered orally as antitubercular agent,4,5 P.A.S.-silicon adduct could also applied in analogous manner.

EXPERIMENTAL

Reagents and analytical techniques

P–amino salicylic acid was of British Pharmaceutical Codex purity and supplied by British Drug Houses Limited. Salicylic and benzoic acids were analar grades obtained from May and Baker Chemical Laboratories. These acids were dried in vacuo over potassium hydroxide prior to use. Tetraethoxy silane was analar grade obtained from Hopkin and Williams and was used as supplied. Benzene, tetrahydrofuran, toluene, and acetone were laboratory reagent grades which were dried over molecular sieve type (4A) before use. Reactions were carried out in a $250cm^3$ three-neck round bottom flask equipped with a reflux condenser, dropping funnel, thermometer and a mechanical stirrer.

Carbon, hydrogen and nitrogen were estimated by the Chemistry department analyst of University of Ife, Ile-Ife. Oxygen was estimated by difference.

The infra-red spectra were recorded in the region $400\text{-}4000cm^{-1}$ with a UNICAM grating spectrophotometer (model S.P. 110) using KBr plates.

Silicon content was estimated gravimetrically as silicon dioxide by the method described by Vogel.[6] Mass spectra were recorded using an A.E.I. Model M.S 12 Mass Spectrometer with gas chromatograph. Since these compounds are not fully soluble in all the organic solvents, the n.m.r. analysis are not carried out.

PROCEDURE

(i) Reaction of Silicon tetrachloride with P-amino Salicylic acid in tetraethoxy-silane.

To a stirred solution of P-aminosalicylic acid (7.657g, 0.05mol.) in tetraethoxy-silane (110ml) was added slowly from a dropping funnel silicon tetrachloride (2.125g, 0.0125mol) in tetraethoxy silane (30ml.) at 25°C under argon. The reaction flask was

heated at constant temperature of 90°C for 7 hours. After this period the white precipitates produced was filtered off and thoroughly washed with acetone and dried in vacuo yield 9.12g (58.31%), m.p. 224-227°C (Decomp).

Found: C,42.63%; H,3.62%, N,6.98%, Si,3.60%, $C_{28}H_{28}N_4O_{12}Cl_4Si$ requires C,42.97%; H,3.58%; N,7.16%; 0,24.55%; Cl,18.16%; Si,3.58%.

Molecular ion at m/e 783. The crystalline compound is slightly soluble in polar organic solvents, insoluble in non-polar solvents but soluble in aqueous acidified solutions:

Reaction of silicontetrachloride with P-aminosalicylic acid in acetone.

To a stirred solution of P-aminosalicyclic acid (7. 655g, 0.05mol.) in acetone (150ml) was added slowly from a dropping funnel silicon tetrachloride (2.124g, 0.0125mol.) in acetone (50ml.) under argon at 25°C On addition of silicon tetrachloride in acetone, a white solid was precipitated out and stirring continued for another 2 hours. The white precipitates were filtered off in a sintered glass funnel, washed several times with acetone and finally dried in vacuo over KOH. The yield was 9.5g, (60.74%), m.p. 227-230°C (Decomp).

Found: C,42.77%; H,3.60%; N,7.09%; Si,3.54%. $C_{28}H_{28}N_4O_{12}Cl_4Si$ requires C,42.97%; H,3.58%; N,7.16%; 0,24.55%; Cl,18.16%; Si,3.58%.

Molecular ion m/e 782. The physical properties of this compound was similar with that of the solid obtained from the reaction of P.A.S. with $SiCl_4$ in tetraethoxy-silane described above.

The infrared spectra of the two solids in nujolmull are identicals which showed very strong and large characteristics NH_3^+ absorption bands between 2800[3] 3000cm^{-1} Other strong NH_3^+ absorptions shown in the spectrum are at 1590 and 1510cm-1 respectively. The other bands are:- the strong νC-O and OH band at 1200cm^{-1}; strong νC=O band at 1695cm-1. very strong νC-O absorption at 1250cm-1, followed by the ν_{as} and ν_{sym}. absorptions of SiO_4 at 809 and 787 cm-1 respectively. Observation showed that the missing absorption bands at 1448, 766 and 1215cm-1 presumably belong to δOH of Carboxylic acid νSiNSi and δSiNH.

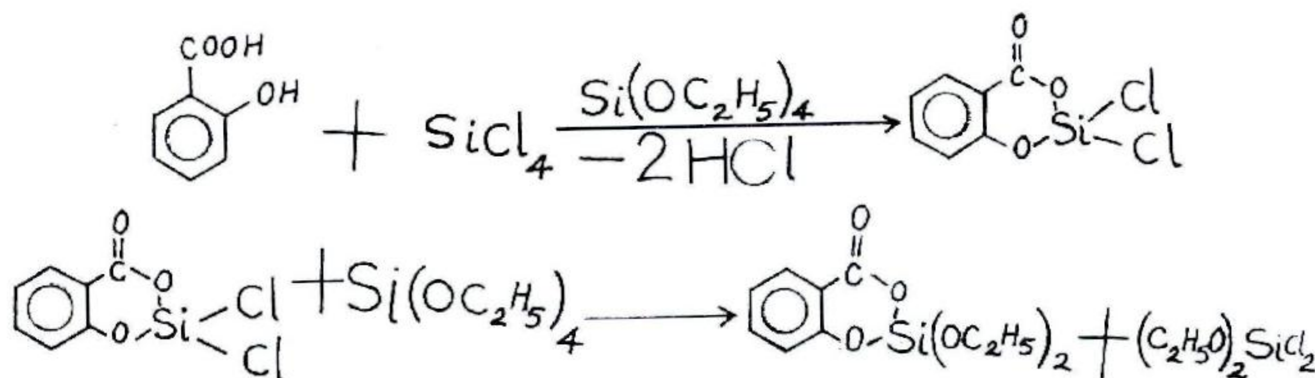
(ii) Reaction of tetraethoxy-silane with P-amino Salicylic acid in benzene.

P-amino salicylic acid (7.657g, 0.05mol.) and tetraethoxy silane (21.6g, 0.104mol.) in benzene (60ml) were placed in a three neck round bottom flask equipped with a reflux condenser and a mechanical stirrer The mixture was heated to boiling and maintained at reflux for 8 hours. After 4 hours ethanol produced from the reaction was continuously distilled off with benzene at 78-80°C. The mixture was allowed to cool then the yellowish-white solid was filtered off, washed several times with acetone and dried in vacuo over KOH. The yield was 9.21g, (57.16), m.p. 199-204°C (Decomp).

Found C,52.10%; H,3.41%; N,8.12%; Si,5.59% $C_{21}H_{17}N_3O_9Si$ requires C,52.17%; H,3.52%; N,8.70%, 0,29.81%; Si,5.80%. The mass spectrum analysis showed the highest molecular ion peak at m/e 483. The pale yellowish-white crystalline compound is insoluble in most of the organic solvents.

Reaction of tetraethoxy-silane with P-amino Salicylic acid in tetrahydrofuran.

P-aminosalicylic acid (3.828g, 0.025mol.) and tetraethoxysilane (21.6g, 0.104mol.) in tetrahydrofuran (70ml.) were placed in a threee neck roundbottom flask equipped with a reflux condenser and a me-



chanical stirrer. The mixture was heated to boiling and maintained at reflux for 5 hours. After 2½ hours, the ethanol produced from the reaction and tetrahydrofuran were continuously distilled off at 65-69°C The light yellowish-white solid left in the flask was filtered off, washed several times with acetone, tetrahydrofuran and finally dried *in vacuo.* The yield was 6.57g, (67.94%). M.P. 198-203°C (Decomp.).

Found: C,52.12%. H,3.49%; N,8.22%; Si,5.53%. $C_{21}H_{17}N_3O_9Si$ requires C,52,17%; H,3.52%; N,8.70%; O,29.81%; Si,5.80%.

Molecular ion peak at m/e 484. The infra-red spectra of the two solids in nujol mull showed strong double absorption bands characteristic of NH_2 at 3450 and 3350cm^{-1} respectively. There is another strong NH-band at 1650cm-1. The large carboxylic acid OH-band absorbed at 3100, while the phenolic OH-band is observed at 1200cm^{-1}. a strong C-O absorption at 1650cm.$^{-1}$ and the SiO_4 and at 830cm^{-1}. Observation showed that the missing absorption bands at 765 and 1213cm-1 can be attributed to a SiNSi and SiNH.

The elemental, infra-red spectra, and mass spectra analyses have shown the two compounds obtained in benzene and tetrahydrofuran media to be identical.

(iii) Reaction of Silicon tetrachloride with Salicylic Acid in tetraethoxy-silane

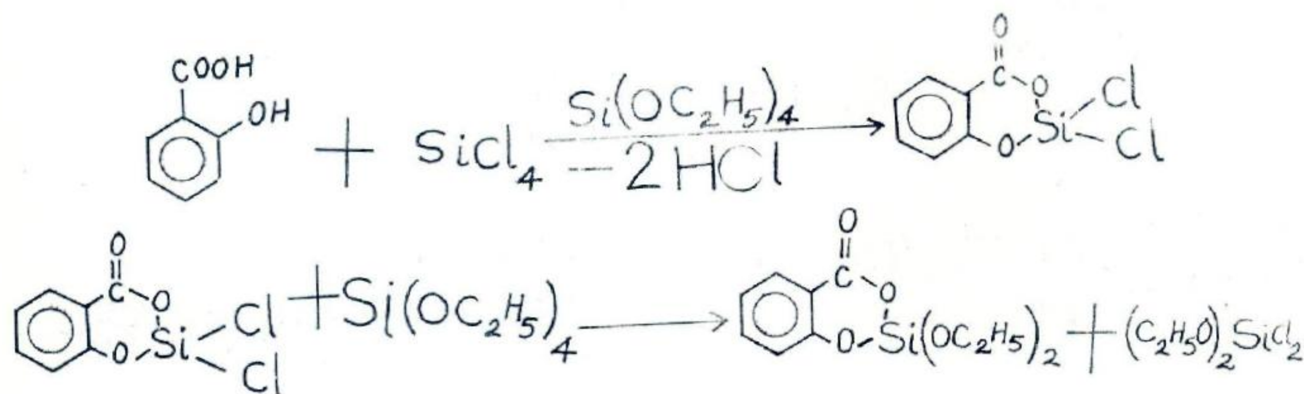
from diethoxydichlorosilane and tetraethoxysilane peaks. The g.c. – mass spectrum showed the molecular ion at m/e 254. The yellow filtrate after distillation under low pressure at 122-126°C/3mm. yielded 5.43g of oily liquid.

Found: C,51.33%. H,5.55%. Si,10.98%

$C_{11}H_{14}O_5Si$ requires C, 51.97%; H,5.51%; 0.31.5% Si,11.02%.

iv. Reaction of benzoic acid with tetraethoxy-silane in toluene

Benzoic acid (6,18g. 0.051mol.) and tetraethoxysilane (22.42g, 0.11mol.) in toluene (80ml.) were placed in three-neck flask equipped with a reflux condenser and a mechanical stirrer. The mixture was heated to boiling and maintained at reflux for 7½ hours. After 4 hours the ethanol produced from the reaction



To a stirred solution of Salicylic acid (6.9g O.05 mol)in silicon tetrachloride (8.5g, 0.05mol.) in tetraethoxysilane (100ml.) under argon at 25°C. Reaction was observed to have started at 25°C before the reaction flask was heated in an oil bath at constant temperature of 130-140°C for 15 hours. The yellow suspension was filtared

The infra-red spectrum of the yellow filtrate showed a strong carbonyl absorption band VC=O at 1765cm-1; the C-H absorptions of the CH3 at 2995s and 2940s cm-1 and the CH2- band at 2915 w. cm^{-1}; and finally the SiO4 bands at 776m and 813cm^{-1} respectively.

The filterate examined by g.l.c. using a column packed with 15% carbonwax on universal support B at 90oC showed the presence of a single product apart was the lowest boiling fraction (78-80oC) followed by toluene at 109-111°C were continuously distilled off. The liquid product obtained was distilled to remove excess of unreacted tetraethoxysilane leaving 8.25g of oil residue, b.p. 128-130oC/4mm. The liquid compound is soluble in benzene and toluene and it can easily be hydrolysed.

Found: C,54 56%; H,6.92%. Si,9.23%.

$C_{13}H_{20}SiO_5$ requires C,54.93%; H,7.04%; 0,28.17% Si,9.86% The g.c. - mass spectrum showed the molecular ion at m/e 285. The infra-red spectrum of the oily liquid showed some characteristic absorption bands, strong CH_3-bands at 2995 and 2960 cm^{-1}, a strong carbonyl C=O absorption at 1720cm^{-1}; the SiO_4 bands at 790m and 803m. cm-1, respectively.

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A New Antibacterial Polyisoprene from Garcinia Kola Heckel Fruit

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ABSTRACT

From Garcinia kola Heckel (Guttiferae) fruits a polyisoprene benzoate (0.9%) was isolated for the first time. The spectal data for the given compound is described. The compound showed antibacterial activity.

INTRODUCTION

Garcinia kola Heckel popularly known as bitter cola is a member of the family Guttiferae and is a native to West Africa(1). It is found under cultivation in Southern part of Nigeria and the native name (Esan) for it is "Adu". The young trees provide chew-stick and the yellow pulp of the fruit and seeds are eaten(1).

We report here on the isolation, structure elucidation and antibacterial property of a new polyisoprene benzoate ester.

MATERIALS AND METHODS

Plant Material

The fruits of bitter cola were purchased locally. The botanical origin was established with the authenticated specimens bearing similar anatomical data in the Herbarium of Nigerian Medicinal plants and West African Flora in the Nigerian Institute for Oil Palm Research.

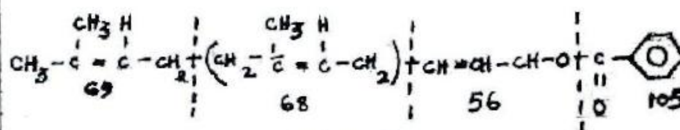
Extraction and Isolation Procedure

Three hundred and thirty-two grams of the dried and powdered pulp of the fruit of Garcinia kola Heckel was extracted with 1 litre of petroleum ether (40-60o) in a Soxhlet apparatus. The extract was concentrated to a low volume at 50o in vacuo. The concentrate on standing in a cool environment yield a brown yellow precipitate (3g). The chloroform solution of the product was spotted on TLC plates. The adsorbent was Kieslgel G (type 6) and solvent system was ehtyl acetate: chloroform (1:9) and (1:4) (Gradient elution). The TLC plates when developed indicated five spots which were designated as A, B. C. D and E, whose Rf values were A=0.92, B=0.85, C=0.8, D=0.45 and E=35. The preparative TLC was also performed with the same absorbent and solvent system. On extraction of the bands in methanol only component B gave appreciable amounts of crystalline product. The product was recrystallised from a mixture of ethanol in water (7:3) to give pale yellow needles, m.p. 107-109o; U.V. max ETOH 255 and 357 nm; I.R. max KBr 3120(S) 2860, 2920. 1650, 1590, 1450, 1420, 1375, 1320, 1270, 1230, 1210, 1180, 1155, 1100. 1080, 980, 920, 800, 790. 770, 730, 690, 655cm-1; NMR signals at 1.6 /dimethyl allyl/ 2.1(HC3-C=C-H) 2.6 (=C-CH2) 3.2 (CH2-CH2) 5.0 (CH-0-COAr), 7.2 and 7.48 (aromatic); M.S. m/e 502 434, 433, 378, 377, 365, 309, 298, 297, 135, 105, 93, 81, 79, 77, 69, 55, 53, 41, 29. (Found: C, 80.73; H, 8.82; Calc. for C35H50O2: C, 81.27; H, 9.16).

RESULTS AND DISCUSSION

After some initial uncertainty we are now fairly confident that the compound isolated from the pulp of the bitter cola fruits is a polyisoprene benzoate. The chain length of the compound was settled by mass spectroscopy and the nature of the isoprene residue was determined by nuclear magnetic resonance spectroscopy. The precise position of all the cis and trans residue is not yet known but is tempting to speculate that the residue are trans from infra-red absorption peaks.

The mass spectrum of the compound shows that the molecular weight is 502 and also the cracking pattern confirms structure of the compound to be farnesy-geranyl propenyl benzoate shown in Scheme 1.



Scheme I

The major fragmentation pattern of I is pictured in Scheme II which account for the important in mass spectrum.

502	434+	+	68 H Transfer
502	433+	+	69
433+	377+	+	56
434+	365+	+	69
or 433+	365+	+	68
Repeated pattern:			
377+	309+	+	68
365+	297+	+	68
	+	–CO	
O	C=O		77+
105+			

In a molecule of this type one would expect the molecular ion to loose C3H4O and also fracture at bonds between adjacent methylene groups(2). This is in fact what happens. The major peaks below m/e =135 are at 41, 55, 69. This 69 peak corresponds to the w-terminal residue coming as a charged fragment.

In the nmr spectrum the resonance in the region at 1.6 is due to dimethyl allyl group. The peaks of 7.2 and 7.4 respectively must be due to 5 protons on the aromatic ring. The absorption band at 5.0 is a sort of a multiplet showing the presence of grouping HC-0-CO-AY. The infra-red spectrum shows the presence of unsaturated linkate linkage at 1650cm-1 overlapping with aryl carbonyl group. Two other bands at about 980 and 920cm-1 arise from out of plane bending motions on vinyl group. The aromatic and the aliphatic C-H stretching frequencies respectively are apparent on the higher frequency and lower frequency side of 3000cm-1 Bands at 1590 and 1450cm-1 are associated with the presence of phenyl group. The methylene bending occurs 1420cm-1. Shown with good resolution the absorption bands at 2960 and 2920cm-1 are assignable to C-H stretchings in methyl and methylene groups respectively. The absorption band at 1375cm-1 is from a symmetrical deformation of the terminal methyl groups. The bands at 1270 and 1230cm-1 are due to C-O stretching vibrations. The absorption bands at 790, 770, 690 and 655cm -1 show the presence of five adjacent protons in the phenyl group.

The highest zones of inhibition were produced by the sample.

Microbiological Screening

Organism	Gram Type	Strain	Zone inhibition for the compound (mm)	Zone of inhibition for the control (mm)
Pseudomonas acruginosa	-	isolated locally	15 + 0.2	1.5
Staphylococcus Aureus	+	ATTCC 10081	14.4 + 0.1	1.5
Bacillus subtilis	+	isolated locally	14.2 + 0.2	1.5
Streptococcus Pneumoniae	+	isolated locally	14.0 + 0.1	1.5
Candida Albicans	+	Isolated locally	14.1 + 0.1	1.5

Quantitative determination of isopropylidene groups(3) in the compound indicated that the molecule contained only one isopropylidene group in agreement with structure I.

Structure I required one terminal methyl in each isoprene unit. A determination showed 0.72-0.73 terminal methyl group for each isoprene unit (4). Since the terminal methyl analysis usually show 0.8 or less for each group, our results are consistent with structure I.

Antibacterial Screening

The compound isolated from the petroleum ether extract was subjected for investigation of antibacterial activity. The sample and the solvent (absolute ethanol) system sterilized previously and 1% w/v placed especially in bores made in nutrient agar seeded with test organisms. The petri-dishes were examined for bacterial growth after twenty-four hours incubation at 37oC and then compared.

The highest zones of inhibition were produced the sample.

Microbiological creening

ACKNOWLEDGEMENT

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The Survival of some Free–Living Microorganisms in Four Frequently Dispensed Liquid Pharmaceutical Preparations

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SUMMARY

The ability of some free-living organisms which were isolated from tap water to surive and if possible proliferate in four different liquid pharmaceutical preparations was examined. These preparations were challenged with suspensions of bacterial cells whose viability over a 28-day period was monitored by taking viable counts at four-day intervals. Some of the organisms not only survived in the preparations throughout the period of the experiment, but also showed appreciable increase in viable count. On the other hand, some of the organisms employed in this experiment lost their viability very rapidly and could not be detected in the preparations after only four days.

INTRODUCTION

Dry pharmaceutical products, including tablets and capsules are not usually free from microbial contamination. Sykes (1969) has shown that whilst these products do not promote microbial growth, the microorganisms present within them can remain viable for long periods. The growth of such contaminants is resumed when conditions become favourable, that is, in the presence of water. In contrast, liquid preparations are not only prone to contamination, they permit the growth of such contaminants. This is why several workers have been able to demonstrate the presence of large bacterial population (sometimes in excess of 1.0×10^6 microorganisms /ml) in several liquid preparations. Sykes (1969) reported the growths of bacterial cells in several liquid preparations while the Pharmaceutical Society Working Party (1977) reported the isolation of up to 1.5×10^8 organisms /ml from several samples of liquid pharmaceuticals. Akinmoji and Ogunlana (1972), following their survey of various pharmaceuticals dispensed in six Nigrian hospitals, also reported the presence of more than 1.0×10^6 organism/ml in many preparations. The presence of such large numbers of microorganisms in liquid pharmaceuticals suggests that these organisms have proliferated within the preparation in question. The implications of these are manifold. The active ingredients of the preparation mya be degraded to give inactive or toxic products (Garrod and O'Grady, 1963) and the instability of formulated preparations may be promoted (Wedderburn, 1964). Mc Call et al (1966) have also shown that the presence of microorganisms in pharmaceuticals is a threat to the health of anyone using such preparations. The raw materials used in the preparation of pharmaceuticals have frequently actedas the initial source of contamination (Syke 1969). Other workers, including Baker (1959), Tennebaum (1965), Tavero et al (1971) as well as Cookson and Horgan (1972) have shown that water from various sources contain large numbers of microorganisms. Water used in the preparation of pharmaceuticals can therefore act as a source of contamination for these preparations.

Lamikanra and Onwudike (1979) have reported the isolation of eleven different types of bacteria from tap water which is the vehicle that is used in the preparation of liquid pharmaceuticals in most Nigerian hospitals. There is therefore a strong possibility that many of these preparations are contaminated. The Organisms which are implicated in this phenomenon may then multiply within the preparation and bring about degradation even in the presence of supposedly sufficient concentrations of preservatives. This possibility is further strengthened by the fact that free-living organisms, such as those found in water are difficult to deal with beacuse of their versatility and increased resistance to antibacterial substances (Tavero et al, 1971). In view of this, it was thought necessary to investigate the ability of the organisms which were isolated from tap water to survive and proliferate in some commonly dispensed liquid preparations.

EXPERIMENTAL/PREPARATION OF BACTERIAL SUSPENSIONS

A loopful of cells from nutrient slope cultures of the microorganism in question was inoculated into nutrient broth and grown in 250 ml conical flasks containing 100 ml medium. The organisms were grown at 37°C for 18 hours after which, the culture was contrifuged at $6.00 \times g$ for 10 minutes in a bench centifuge (MSE, Manor Royal, Sussex, England). The pellet obtained was washed twice with sterile distilled water and finally resuspended in enough sterile distilled water to give a viable count of approximately 1.0×10^5 organisms/ml. Eight of the eleven types of organisms earlier isolated from tap water (Lamikanra and Onwudike, 1979) were used in this experiment.

PHARMACEUTICAL PREPARATIONS

The liquid preparations used in this study included Magnesium trisilicate BPC, potasium citrate BPC, ipecacuanha mixture BPC. These mixtures were employed because a preliminary survey showed that they

are the most fequently dispensed liquid pharmaceutical preparations in hospitals around Ile-Ife, Nigeria.

The Mixtures were prepared as directed by relevant British Phrmaceutical Codex (BPC, 1975) monographs. Prior to preparation, the various liquid ingreidients were sterilised by autoclaving at 115°C for 30 minutes. In the case of volatile substances these were autoclaved in sealed ampoules to prevent evaporation. Powders were sterilised by heating in a hot air over at 115°C for 1 hour. Following this, the ingredients were aseptically **compounded** under a laminar flow cabinet (John Bass Limited, Sussex, England). A 100 ml portion of each preparation was dispensed into a glass stoppered bottle and challenged with 1 ml of thebacterial suspension prepared as earlier described. Each preparation therefore contained approximately 1.0×10^3 micro-organisms/ml. The inoculated preparations were then incubated at room temperature (abut 27oC) over a period of 28 days.

SAMPLING

From the incubated preparation, duplicate 1 ml portions were removed at 4-day intervals, serially diluted in Ringers solution and 1 ml of the final dilution was transferred into each of four sterile petridishes to which 20 ml of molten nutrient agar was then added. The plates were throughly mixed, allowed to set and then incubated at 37°C for 48 hours after which the number of colonies in each plate was counted and the viable count calculated from figures obtained. Samples of preparations to which 1 ml of sterile water was added were treated in an identical manner and used as controls. No organisms were detected in any of the control samples throughout the 28-day period of the experiment.

RESULTS AND DISCUSSION

The results are shown in Figures 1-6 from which it can be seen that most of the organisms employed proliferated very rapidly in each of the four preparations. The organisms employed in this study included four Gram-positive cocci. The two Gram-positive rods not only survived but proliferated within each of the four preparations used. A substantial number of these organisms were isolated from each preparation throughout the period of study (Figures 1 and 2). Of the four Gram-negative rods **from** in the challenge, two died off in all the preparations within 8 days (Figure 4), but the other two survived and proliferated within the preparations (Figure 3 and 5). The two Gram-positive cocci employed were not detectable in the preparations shortly after they were incubated except in the case of the organisms which seemed to have become adapted to conditions in the ipecacuanha and ammonia mixture paediatric BPC (Figure 6). The situation was such that it proliferated within the preparation towards the end of the 28-day study period.

On the whole, the observations are in broad agreement with earlier work in this field. Westwood and Pin-lin (1972) have, for example, reported the survival of various organisms including E coli, Ps. aeruginosa and S. aureus in both Magnesium trisilicate and Potassium citrate mixture over period exceeding 7 days. Furthermore, Sykes (1969); as well as Akinmoji and Ogunlana (1972) were able to isolate large numbers of organisms from many different preparations presumably becasue of the ability of these microorganisms to survive and grow within the preparations considered.

It is interesting to note that organisms were able to survive and even multiply in the various preparations even though each of these preparations contained chloroform water which is supposed to act as a preservative. The inability of chloroform water to prevent bacterial growth in these preparation might have been caused by the ability of the various microorganisms to utilise chloroform water as a source of carbon and energy. Examples of this phenomenon have been provided by Bock (1967) who reported the ability of Aspergilus niger to degrade benzoic acid, and also by Burdon and Whitly (1967) who isolated Ps. cepacia from various antibacterials solutions including chlorhexidine and certrimide. Brown (1971) has also demonstrated that peppermint water is virtually a selective medium for Ps. pyocyanea. It is also worth remembering that free living organisms are generally resistant to the activity of anti-bacterial agents becasue of the characteristic structure of their cell envelope (Brown, 1975).

From the results obtained, it can be seen that some of the organisms employed in the test failed to survive when inoculated into the different preparations used. The inability of these organisms to survive may be attributed to the antibacterial activity of the chloroform water which was incorporated into the preparation. On the other hand, it is possible that these organisms failed to survive in the preparations because they were unable to utilise any of the ingredients of these preparations as a source of carbon and energy For such organisms, the inclusion of preservatives in pharmaceutical preparations is unnecessary, the internal environment of pharmaceuitcals being sufficiently hostile to prevent their survival (Westwood and Pancholi, 1974).

The undesirability of having grossly contaminated liquid pharmaceuticals has earlier been discussed (Lamikanra and Onwudike, 1979). The results of this investigation show that free-living bacteria can remain viable in pharmaceutical preparations over long periods. It is therefore important to ensure that all the raw materials used in the preparation of pharmaceuticals are free large bacterial population. Special care is required in hospital pharmacies which is why Baird, Parks and Awad (1977) have suggeted the introduction of an environmental control programme to minimise the possibility of contamination of hospital preparations. In places where such program-

mes cannot be introduced it may be desirable to routinely incorporate adequate concentrations of preservatives into all hospital preparations. Such preservatives must however be rigorously tested so as to ensure that they are effective under the conditions of use.

Figures 1 – 6

The survival patterns of some free living microorganisms in

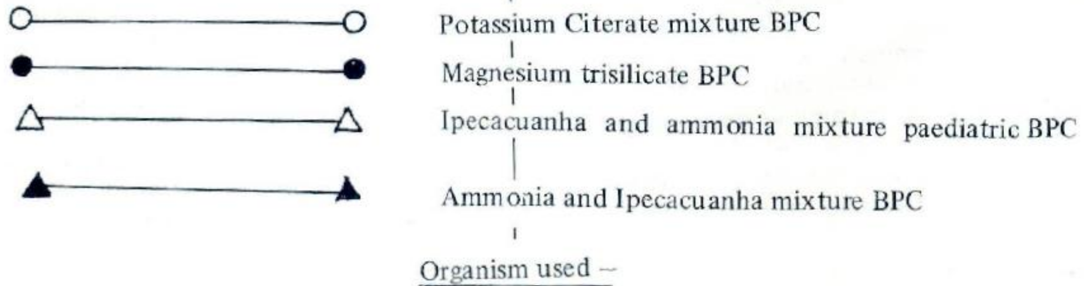

Fig. 1. Gram positive rod
Fig. 2. Gram positive rod
Fig. 3. Gram negative rod
Fig. 4. Gram negative rod
Fig. 5. Gram negative rod
Fig. 6. Gram positve coccus

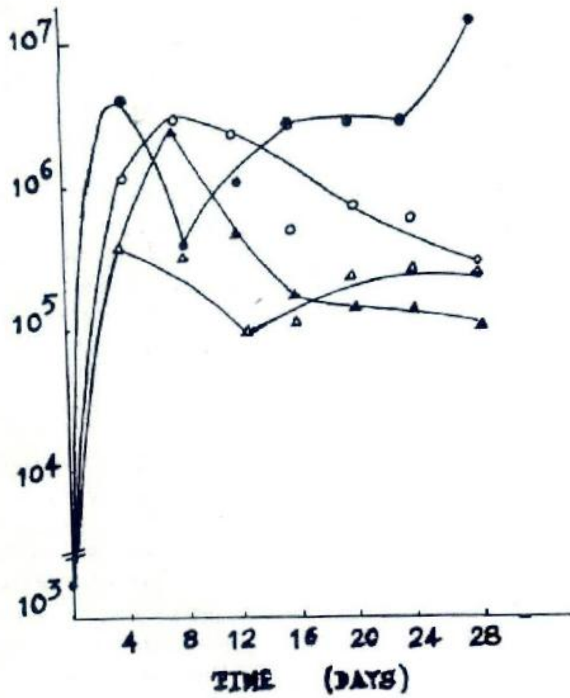

Fig 1

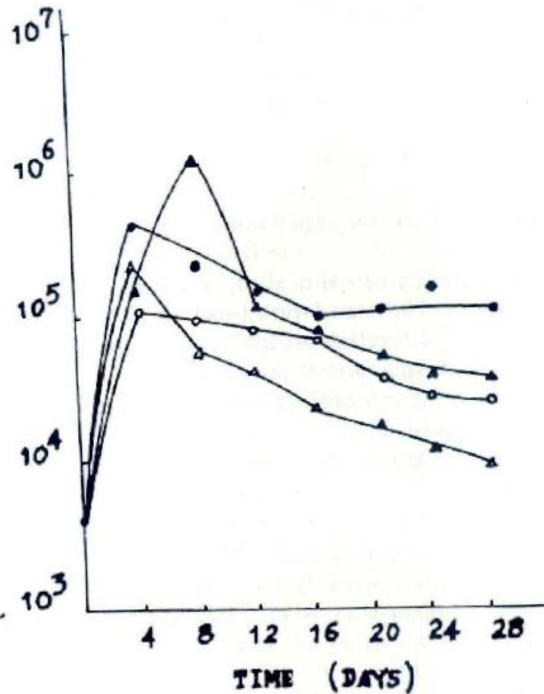

Fig 2

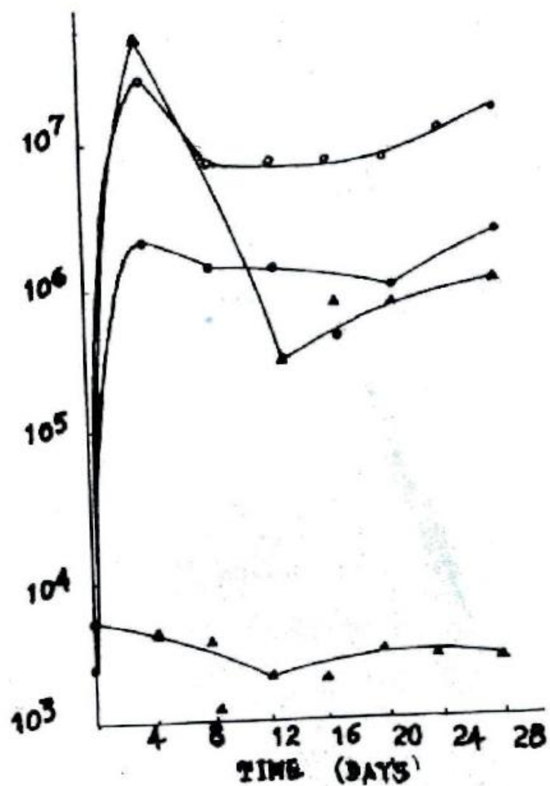

Fig. 3

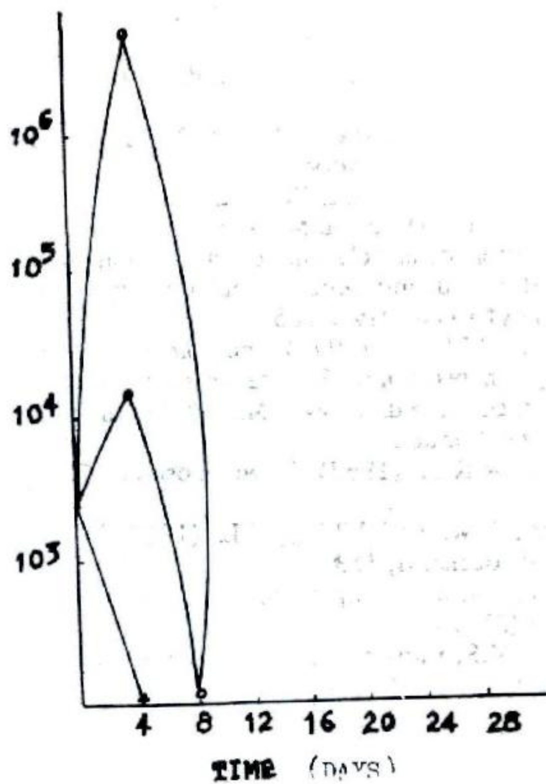

Fig. 4

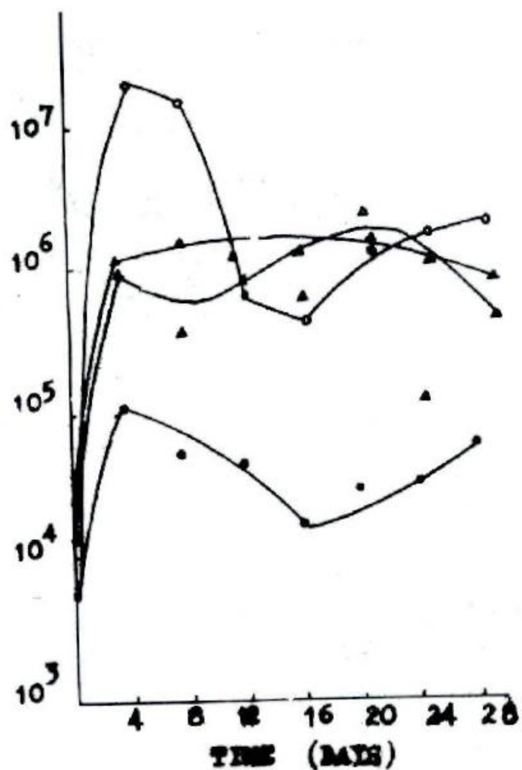

Fig. 5

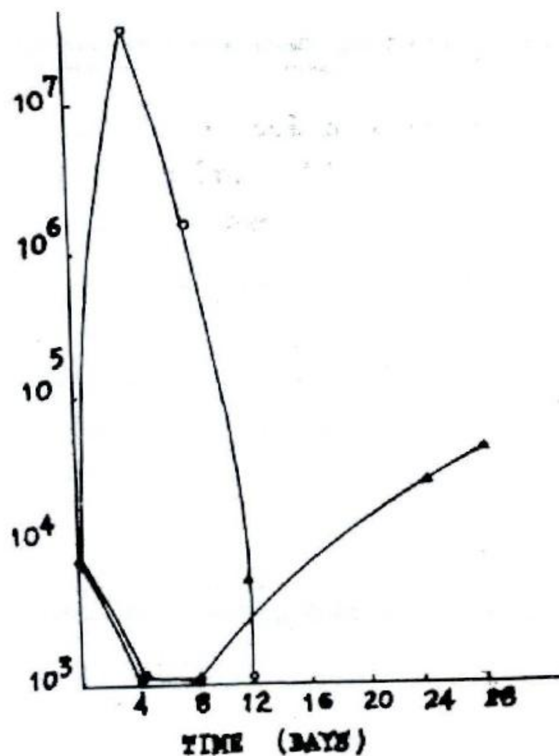

Fig. 6

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