Studies on the Condensation Route to Bisflavanoids

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ABSTRACT

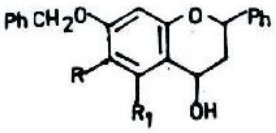
The products of several coupling reactions involving many flavan-4-ols and 7-hydroxy-5-methoxyflavan under various conditions were evaluated by T.L.C., I.R., UV/VIS., P.M.R. and M.S. On the basis of the findings, the conditions for maximising the yield of 4,8′-bisflavans which are important intermediates in the synthesis of some vegetable astringents have beer. outlined.

INTRODUCTION AND DISCUSSION

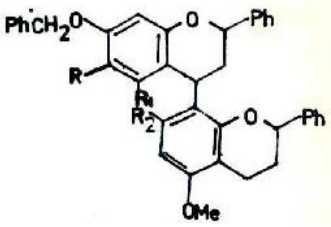
Freudenberg and Maitland(1) were the first to postulate a condensation mechanism for flavanoids that involves the elimination of water. Brown et. al(2-5) investigated this postulate by condensing various benzyl alcohol (including flavan-4-ols) with several phenols in ethanolic hydrogen chloride and recorded varying degrees of success. Geissman and Yoshimura(6) using dioxane-water solution that was approximately O.IN in HCl also reported successful condensation at O°C of equimolar amounts of flavan-3, 4-diol with phloroglucinol and (.)-catechin. These authors reported the isolation of two major products formed in roughly equal amounts during each condensation. Their products could not be crystallised. Later, Jurd and Lundin(7) reported the successful condensation of 5,7,3′-tetramethoxy-flavan-3, 4-diol with phloroglucinol and resorcinol. Their condensation reactions were carried out in the presence of a large excess of phloroglucinol and resorcinol and in either dilute aqueous acetic acid or in aqueous buffer solutions (pH3.2). The expected products were obtained directly as crystals in yields of up to 90% and in each case only a single condensation product was formed.

Thus, widely differing results in products and yields were reported by different workers even when similar methods of preparation were employed and the duration of the experiments was sometimes up to 7 days(2-5). During synthetic studies aimed at synthesising dracorubin, a complex anhydropyranol bisflavan base by the author and co-worker,(8) this problem was repeatedly encountered and sometimes the yield after work-up was too low for this method to gain synthetic importance. This experience necessitated a careful study of the coupling reactions with a view to maximising the yield of the bisflavan in the shortest possible time. The significance of this investigation lies in the fact that the acid catalysed coupling of flavan-4-ols to 4-unsubstituted flavans is the only proven synthetic route so far to 4,8′-bisflavans.(8-10)

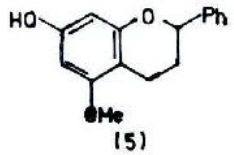
Accordingly the products of several coupling reactions involving flavan-4-ols and a 4-unsubstituted flavan under varying conditions of molecular ratios, temperature, duration of experiment and total weight of reactants relative to volume of acid were evaluated by TLC, IR, UV/VIS, P.M.R. and M.S. All the flavan-4-ols (1-4) and 7-hydroxy-5-methoxyflavan (5) used were synthesised by standard procedures (8,9). In the first set of coupling trials a mixture of 7-benzyloxy-5-methoxyflavan-4-ol(1) and 7-hydroxy-t-methoxyflavan (5, 3 times in excess of the calculated equivalent amount)[7] was treated with 6% v/v aqueous acetic acid at 50°C. The ratio of the volume of dilute acid to the combined weight of reactants was maintained at about 47mls: 0.5g. The crystalline products obtained after 15hr at room temperature were evaluated by the various techniques enumerated. TLC showed four distinct bands. (Rfs 0.22, 0.50, 0.63, 0.68 respectively). Products obtained for bands 1-4 were 40mg, 40mg, 45mg and 25mg respectivelyusing 150mg. crude product.



(1) R = H, R_1 = OMe
(2) R = CH_3, R_1 = OMe
(3) R = H, R_1 = OTs
(4) R = H, R_1 = OH



(6) R = H, R_1 = OMe, R_2 = OH
(7) R = CH_3, R_1 = OMe, R_2 = OH
(8) R = H, R_1 = OTs, R_2 = OH
(9) R = H, R_1 = OH, R_2 = OH
(10) R = H, R_1 = ACO, R_2 = ACO
(11) R = H, R_1 = OMe, R_2 = ACO
(12) R = CH_3, R_1 = OMe, R_2 = ACO



(5)

Band 1: The isolated compound crystallised in clusters of long slender needles (m.p. 93°C, M|,256) from aqueous acetic acid. M.m.p. with (5), 93°C. The compound analysed correctly for $C_{16}H_{16}O_4$. The m.p., molecular weight, P.M.R. I.R., Rf and elemental analytical data were identical with those of 7-hydroxy-5-methoxy-flavan(5) and band 1 was therefore identified as the unreacted flavan(5).

Bland 2: The separated compound crystallised in colourless needles (m.p. 109°C, M.362) from peroxide-free diethyl ether and the crystals gave correct elemental analysis for $C_{23}H_{22}O_4$. From comparative T.L.C., M.P., molecular weight, P.M.R., I.R. and elemental analytical evidence, band 2 was identified as unreacted 7-benzyloxy-5-methoxy-flavan-4-ol(1).

Band 3: On isolation furnished a snow-white amorphous powder (mp.95-108°C, decomp.) which resisted crystallisation and was thus characterised as the acetate. Acetylation with acetic anhydride and pyridine gave a product which crystallised from absolute ethanol in colourless micro-needles (mp.117°C). Infrared spectrum in nujol included peaks at 1760 (acetate carbonyl): 1600 (aromatic) and 1205cm^{-1} (arylacetate). PMR gave signals at -2.65(15H, three phenyl), -3.70 (S,1H), 3.82 (M,2H, -4.9(S,2H, a benzylic methylene), -5.0 (M, 3H, three benzylic methines), -6.24-6.47 (6H, two methoxyl), -7.30-8.9 (M,9H, three methylene groups and one acetate methyl) all integrating to 38 protons. High resolution mass spectrometry showed a molecular ion at m/e642. Elemental analysis established the molecular formula $C_{41}H_{38}O_7$. Thus the elemental analytical and the spectral data are consistent with this compound being formulated as the acetate (11) of a bisflavan and band 3 is therefore the bisflavan (6).

Band 4: on isolation was a cream-coloured resin-like powder, gummy when hot, hard and brittle when cold. P.M.R. showed only mounds. Acetylation afforded an intractable product. It was therefore concluded that this band was a deterioration product, possibly polymerised.

More than half the flavan and flavanol were recovered unreacted and the ratio by weight of the bisflavan (band 3) to the deterioration product (band 4) was about 2:1 even though the respective spot areas on TLC were roughly equal.

Increasing the volume of dilute acid whilst keeping the weights of the reactants constant gave products with less of the unreacted flavan (band 1), flavanol (band 2), deterioration product (band 4) and more of the bisflavan (band 3).

It became clear from these findings that the flavanol cannot readily couple with itself under the conditions of the experiment hence the consistent recovery of unreacted flavanol. The same was true of the flavan. The use of either of them in excess was therefore considered unnecessary. Further condensation experiments were therefore carried out with equimolar amounts of the flavan and flavanol. Using equimolar amounts of flavan and flavanol, a quantitative condensation was obtained when the ratio of volume of dilute acid to combined weight of reactants rose to 526ml:0.5g. The product of this condensation was a snow-white amorphous powder that resisted crystallisation and gave one spot on TLC. Its infrared spectrum included peaks at 3450 (hydroxyl), 2845 (methoxy1) and 1600cm^{-1} (aromatic). The P.M.R. spectrum in deuteriochloroform indicated the presence of three pheny1 groups at T2.65–2.78 (15H), two aromatic protons at T3.78, one aromatic proton singlet at T4.05, one hydroxy1 proton singlet exchangeable with D_2O at T4.35, a benzylic methylene singlet at T5.0 (2H), three benzylic methine protons at T5.10, two methozy1 methy1 groups at T6.35 (6H) and three methylene groups at T7.35–7.80(6H) all integrating to 36 protons. After allowing for half a molecule of water, the elemental analysis agreed with the formula $C_{39}H_{36}O_6.\frac{1}{2}H_2O$.

The inclusion of a molecule of water was found necessary in the case of previous comparable compounds10-13, because bisflavans hold water tenaciously.

High resolution mass spectrometry showed a molecular ion at m/e600 thus unambiguously indicating the molecular formula. $C_{39}H^{36}O_6$ and correlating the elemental analytical data. Thus, the elemental analytical and spectroscopic data are consistent with this compound being formulated as the bisflavan, 7-benzyloxy-5-methoxy-(4,8′-(7′-hydroxy-5-methoxy flavan))-2-phenylbenzopyran (6). Acetylation of (6) gave the acetate (11) which was identical with the acetate prepared from Band 3.

Table 1 shows the volumes of dilute acid which produced a quantitative yield of the bisflavan in each coupling reaction. Volumes of dilute acid much less than that produced the four bands already described.

From the results, there is now no doubt that the main factor determining the purity and quantity of the bisflavan formed during these condensation reactions is the volume of the dilute acid.

TABLE 1

Reactions	Flavan 4–ol.	Flavan	Bisflavan formed	Vol.: Combined Wt. ratio for successful condensation
1	1	5	6	526ml : 0.5g.
2	2	5	7	2120ml : 0.5g.
3	3	5	8	1700ml : 0.5g.
4	4	5	9	2125ml : 0.5g.

In each of the coupling reactions in table 1 all the resultant bisflavans, except (8) which was unstable, gave the expected spectral results and analysed correctly for the required C, H and O. The difference in the volume of dilute acetic acid required for a quantitative coupling is believed to be related to the solubility of the reactants. The crysalline flavan (5) is not very soluble in many solvents including acetic acid. It also crystallises readily from dilute acetic acid in needles and might be responsible for the crystals obtained when incomplete coupling takes place. Since however its quantity in these couplings is fairly constant, the solubility of the flavan-4-ol then becomes the determinant factor. The 6-methyl-flavanol (2) is very much less soluble in polar acetic acid than the nor-compound (1) due to the significant contribution of the methyl group to the hydrocarbon nature of that compound. 5-Hydroxyflavanol (4) requires about the same volume of acetic acid as (2). The reason for this is the reduction in the solubility of (4) due to intramolecular hydrogen bonding. This is shown in the IR. Spectrum of (4) which includes a strong absorption at 3450cm-1. The 5-tosyloxyflavanol (3) required about 3 times the volume of dilute acid used in the coupling of (1) because of the very much larger molecular weight of the tosylate and the associated reduction in solubility. Coupling at temperatures far beyond 50°C gave more of the resinous product as shown by the products obtained at 80°C. Provided a sufficient volume of acid is used coupling occurs readily at 50°C but a very large volume of dilute acid gives a very fine particulate bisflavan which is usually very difficult to filter.

EXPERIMENTAL

Infrared spectra were measured on a Perkin-Elmer model 157G grating spectrophotometer, UV/VIS spectra on a Hitachi Perkin-Elmer model 124 double beam spectrophotometer, PMR spectra on a Perkin-Elmer model R-12A spectrometer and Mass Spectra on AE1 MS902 double-focussing Mass spectrometer.

Condensation Experiments:

The condensation reactions were carried out as described below, unless otherwise stated.

The required weight of flavan-4-ol and the calculated weight of 7-hydroxy-5-methoxyflavan were mixed and dissolved in the calculated volume of glacial acetic acid. With the resultant solution maintained at 50°C, a volume of water also at 50°C calculated to give 6% aqeous acetic acid solution was added slowly (30-60 sec.) to the glacial acetic acid solution. The resultant suspension was mechanically stirred for 5 minutes before being set aside at ambient temperatures for 15 hours. Subsequent filtration afforded the solid product used for the studies.

TLC—was on kieselgel GF 254 (Merck), 0.3mm thick plates using chloroform as solvent.

Condensation trials:

7-Benxyloxy-5 methoxy (4,8'(7'-hydroxy-5'-methoxyflavan))-2-phenyl-benzopyran (6).

7-Benzyloxy-5-methoxyflavan-4-ol (1,50mg; 100mg, 200mg, 1.0g.) was treated respectively with 7-hyroxy-5-methoxyflavan (5.106mg, 212mg, 424mg, 2.12g (3 times equimolar amount)) using 14.9ml, 29.7ml, 60.4ml and 300ml respectively of dilute acetic acid. Creamy white slender needles mixed with amorphous solid was filtered 15 hours later in yields of 156mg, 312mg, 624mg and 3.12g. respectively. TLC consistently gave four bands in roughly the same proportions. The bands were eluted with diethyl ether. 150mg subjected to TLC gave 40mg, 40mg, 45mg and 25mg, respectively of bands 1-4.

Band 1: White powder crystallising in clusters of long colourless slender needles from aqueous acetic acid. Mp.93°C, M.256. From IR., PMR, MS, elemental analytical data, m.p. and m.m.p., this band was identical with 7-hydroxy-5-methoxyflavan (5).

Band 2: White powder crystallising from peroxide free ether in colourless needles. Mp.108-109°C, M.362. From IR, UV/Vis., PMR, MS, elemental analytical data, m.p. and m.m.p., this band was identical with 7-benzyloxy-5-methoxyflavan-4-ol (1).

Band 3: Snow-white amorphous powder that could not be crystallised and was therefore characterised as the acetate. 200Mg of the white powder on treatment with acetic anhydride (2ml) and pyridine (10 drops) in the usual way furnished the acetate (204mg) which crystallised from absolute ethanol in colourless micro-needles. M.P. 117-118°C.

Found: C,76.5; H,6.1; $C^{41}H^{38}0^{7}$ requires C,76.6; H,6,1%;

P.M.R. signals at -2,65(15H), 3,7(S,1H), 3,82(M,2H), 4,9(S.2H), 5.0(M,3H), 6,24-6.47(M,6H), 7.30-8.9(M,9H).

νMax. (Nujol), 1760 (acetate carbonyl); 1600 (aromatic); 1205cm^{-1} (aryl acetate). Mass spectrum signals at m/e relative abundance) 642(48), 641(96), 610(26), 599(17), 495(24), 344(31), 266(17), 253(24), 117(19), 91(100),

Band 4: Cream coloured resin-like powder, P.M.R. showed only mounds. Acetylation afforded an intractable product which was abandoned at that stage.

Effect of duration of experiment:

7-Benzyloxy-5-methoxy flavan-4-ol (1,50mg, 100mg) was respectively treated with 7-hydroxy-5-methoxyflavan (5,106mg, 212mg, 3 times calculated equivalent amount) using 14.9ml and 29,7ml respectively of dilute acetic acid. The product was evaluated in the usual way after 72hrs, 60hrs, 48hrs, 24hrs and 5 minutes of reaction. TLC of successive products gave the usual 4 bands on TLC.

Effect of temperature:

7-Benzyloxy-5-methoxyflavan-4-ol (1,50mg) was treated with 7-hydroxy-5-methoxyflavan (5,106mg) using 14,9ml of dilute acetic acid and at a temperature of 80°C. Reaction mixture was stirred for 5 min and then set aside at room temperature. Filtration gave a light yellow solid, TLC showed the usual 4 bands but band 4 was twice as much as that from experiments performed at 50°C, and there was a general reduction in the quantities of bands 1-3 isolated.

Effect of Volume of Dilute Acid:

A mixture of equimolar quantities of 7-benzyloxy-5-methoxyflavan-4-ol (1,200mg) and 7-hydroxy-5-methoxyflavan (5,142mg) was in separate experiments treated in the usual way at 50°C with dilute acetic acid (40ml, 80ml and 300ml). The product from each volume was filtered after 72hrs, 40hrs, 15hrs and 5 minutes of reaction and evaluated in the usual way.

Products from 40 and 80ml Dilute Acetic Acid:

Cream coloured products were obtained. TLC of each product exhibited the usual 4 bands, duration of reaction notwithstanding.

Products from 180ml Dilute Acetic Acid:

Filtration afforded in each case an amorphous snow-white granular powder 342mg. Even after 5 minutes of reaction TLC exhibited a single band Rf 0.63. The product could not be crystallised and was used directly for the determinations. M.p. 100-110°C (decomp). shrinks at 80°C. Found: C,76,9; H,6.1: $C_{39}O_{36}$. $\frac{1}{2}H_2O$ requires C,76.8; H,6.1%. P.M.R ($CdCl^3$) signals at -2.65-2.78(15H), 3.78(2H),4.05 (S,1H), 4.35(S,1H), exchanges with D_2O), 5.0 (S,2H), 5.10(M,3H), 6.35(M,6H), 7.35-7.80(M,6H). "Max . (KBr), 3450 (hydroxy1); 2845 (methoxy1), 1600cm^{-1} (aromatic). Max. (EtOH), nm(logs) 210(4.90), 236 sh (4.35), 270(3.43). Mass spectrum, m/e (relative abundance): 600(21), 465(7), 344(48), 256(41), 253(36), 152(41) 104(20), 91(100).

Acetylation afforded a crystalline product whose spectral and other properties were identical with those of the acetate (II).

Products from 300ml dilute acetic acid:

Filtration furnished amorphous snow-white very fine powders. TLC of all products showed one band. Rf 0.63. Particles were very fine and difficult to filter. Spectral properties were identical with those of (6).

7-Benzyloxy-5-methoxy-6-methyl (4,8'-(7'-hydroxy-5'-methoxy-flavan))-2-phenylbenzopyran (7). A mixture of equimolar quantities of 7-benzyloxy-5-methoxy-6-methyflavan-4-ol(2,200mg) and 7-hydroxy-5-methoxyflavan (5,136mg) was treated in seperate experiments with dilute acetic acid (80ml, 400ml, 900ml, 1,500ml) at 50°C. The products were filtered after 12 hours, and evaluated in the usual way.

Products from 80 and 400ml dilute acetic acid:

Cream coloured solids were obtained. TLC revealed the usual 4 bands. Bands 1 and 2 corresponded to unreacted flavan (5) and flavanol (2) respectively. Band 3 was the bisflavanoid (7) and band 4 the resin-like product.

Product from 900ml dilute acetic acid:

A granular white powder of 7-benzyloxy-5-methoxy-6-methyl 4,8'(7'-hydroxy-5'-methoxyflavan) –2–phenylbenxopyran (7,336mg) was obtained by salting out with sodium chloride (9g), M.p. 116-127°C, (decomp). The product exhibited a single band on TLC and resisted crystallisation. Found: C,77,1 H,6.1 $C_{40}H_{38}O_6,\frac{1}{2}H_2O$ requires C,77.0; H,6.3%. Max. (CCl_4), 3460 (free hydroxy1), 2850 (methoxy1), 1610cm^{-1} (aromatic). Max, (EtOH), nm (logs) 210(4.91), 235sh (4.31), 280 (3.66). PMR.($CDCl^3$), signals at -2.63 (S,15H, aromatic) 3.60(1H, aromatic) 4.0(S,1H, aromatic), 4.54(S,15H, aromatic) 3.60(1H, aromatic), 4.0(S,1H, aromatic), 4.54(S,1H), exchanges with D^2O, hydroxyl), 4.96 (M,5H,(2H, benzylicmethylene; 3H, benzylic methine)) 6.2-6.52 (M,6H, two aryl methoxyl), 7.3-8.4 (M,9H, (6H, three methylenes; 3H, aryl C-methyl)). Massspectrum, m/e (relative abundance); 614(37), 613(81), 480(25), 359(32), 358(39), 267(56), 105(18), 91(100).

Acetylation afforded a product (12) which crystallised from absolute ethanol as off-white prisms. M.P. 75-78°C, M.656.

Product from 1500ml of dilute acetic acid:

Very fine amorphous white powder, difficult to filter even after addition of sodium chloride. TLC revealed a single band. M.p. 114-125°C. Spectral and elemental analytical data were identical to those of the bisflavan (7).

7-benxyloxy-5-tosyloxy-(4,8'-(7'-hydroxy-5'-methoxyflavan))-2-phenylbenzopyran (8). A mixture of equimolar amounts of 7-benzyloxy-5-tosyloxyflavan-4-ol(3,200mg) and 7-hydroxy-5-methoxyflavan (5,101.08mg) was treated (18h) in seperate experiments with dilute acetic acid (100ml., 300ml, 720ml., 1200ml), at 50°C. In each case about 300mg of a crystalline solid was filtered. TLC was carried out soon after filtration. The 7-benzyloxy-5-tosyloxyflavan-4-ol used was freshly prepared since it is unstable. Products from 100 and 300ml dilute acetic acid were creamish crystalline solids giving 4 bands on TLC. Band 1 and 2 corresponded to unreacted flavan (5) and flavan-4-ol (3) respectively. Band 3 was the bisflavanoid (8) and band 4 the resinous product.

Product from 720ml dilute acetic acid:

White crystalline product which gave one band on TLC. M.p. 214°C (decomp). Unstable on keeping for up to 24 hours at room temperature. It could not be recrystallised. Satisfactory elemental analysis was impossible for the above reasons. Spectral data strongly indicated the bisflavan (8).

Product from 1200ml dilute acetic acid:

Very fine white powder. Difficult to filter. It gave a single band on TLC. M.p. 212°C (decomp). The spectral data strongly indicated the bisflavan (8).

7-Benxyloxy-5-hydroxy-(4,8'-(7'-hydroxy-5'-meth-

oxyflavan) -2-phenyl benzopyran (9). A mixture of equimolar quantitative of 7-benzyloxy-5-hydroxy flavan-4-ol(4,200mg) and 7-hydroxy-5-methoxy-flavan (5,148mg) was treated (18h) in separate experiments with dilute acetic acid 400ml, 900ml, 1500ml), at 50°C.

Product from 400ml dilute acetic aid:

Cream coloured solid. TLC showed 4 bands. Band 1 and 2 were identical with flavan (5) and flavanol (4) respectively. Band 3 was the bisflavan (9) and Band 4 the resinous product.

Product from 900ml dilute acetic acid:

Snow-white granular powder of 7-benxyloxy-5-hydroxy-(4,8'-(7'-hydroxy-5'-methoxyflavan))-2-phenylbenzopyran (9,348g) which showed a single band on TLC., and could not be crystallised. In consequence measurements were made on the dried amorphous powder. M.p. 91°C.

Found: C,76.8; H,5.8. $C^{38}H^{34}0$ requires C,76.6; H,5.9%. Max: (ccl^4), 3470 (hydroxyl, Intra-molecularly H-bonded), 2850 (methoxyl), 1620 and 1585cm^{-1} (aromatic). P.M.R. ($CDCl^3$) signals at -2.65 (S,15H), 3.90(M,2H), 4.00(S,1H), 4.95(M,3H), 5.00(S,2H), 5.32 (Mound, 2H, exchanges with D^20), 6.25(S,3H), 7.33(M,2H), 7.80(M,4H).

The acetate crystallised from methanol-diethylether (20:1) in off-white prisms. M.p. 114-118°C. Found: C,75.4, H,5.8.$C^{42}H^{38}0^8$ requires C,75.2; H,5.7%.

The product from 1200ml was a very fine powder which was difficult to filter. It gave one spot on TLC. The elemental analytical and spectral data showed that the product was the bisflavan (9).

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