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A CONVENIENT SYNTHESIS OF 3,5,7-TRIHYDROXY-8-METHOXY FLAVONE, PRUDOMESTIN AND LIMOCITRIN

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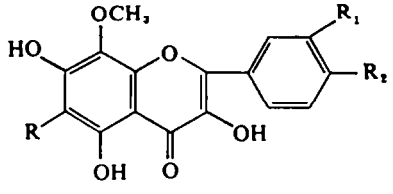
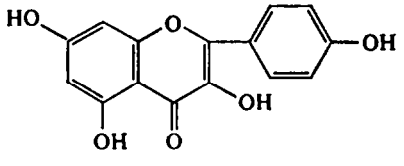
Abstract—The selective demethylation of the 5-methoxyl group of 3-hydroxy-5,8-dimethoxyflavone derivatives could not be carried out by known methods. The synthesis of 8-methoxy compounds has been achieved indirectly by the application of oxidative demethylation using nitric acid. This technique yielded 3,5,7-trihydroxy-8-methoxyflavone, prudomestin (3,5,7-trihydroxy-8,4′-dimethoxyflavone) and limocitrin (3,5,7,4′-tetrahydroxy-8,3′-dimethoxyflavone).

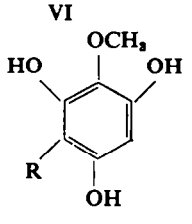
AMONG the naturally occurring 8-methoxyflavonols with the 3,5,7-trihydroxy structure are tambuletin[1] (I), prudomestin[2] (II), limocitrin[3] (III), limocitrol[4] (IV) and isolimocitrol[4] (V). Tambuletin was synthesized[5] from kaempferol (VI) and recently, using dimethoxyphloroglucinol (VII), Dreyer *et al.* synthesized limocitrol.[6] In the same paper[6] a synthesis of limocitrin starting from iretol (VIII) has also been reported. A possible point of ambiguity in this synthesis will be discussed later. The present paper deals with the synthesis of prudomestin and an unambiguous synthesis of limocitrin. In exploratory experiments the synthesis of a simpler member viz., 3,5,7-trihydroxy-8-methoxyflavone has been achieved.



I R = R_1 = H; R_2 = OH
II R = R_1 = H; R_2 = OCH_3
III R = H; R_1 = OCH_3; R_2 = OH
IV R = R_1 = OCH_3; R_2 = OH
V R = R_2 = OCH_3; R_1 = OH



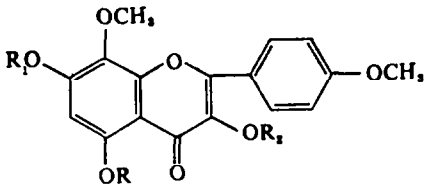
VI



VII R = OCH_3
VIII R = H

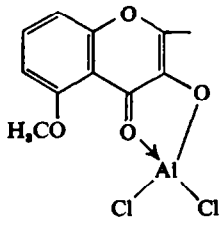
[1] K. J. Balakrishna and T. R. Seshadri, *Proc. Ind. Acad. Sci.* **25A**, 449 (1947).
[2] G. R. Nagarajan and T. R. Seshadri, *Phytochem.* **3**, 477 (1964).
[3] R. M. Horowitz and B. Gentili, *J. Org. Chem.* **26**, 2899 (1961).
[4] B. Gentili and R. M. Horowitz, *Tetrahedron* **20**, 2313 (1964).
[5] K. J. Balakrishna and T. R. Seshadri, *Proc. Ind. Acad. Sci.* **26A**, 234 (1947).
[6] D. L. Dreyer, S. Tabata and R. M. Horowitz, *Tetrahedron* **20**, 2977 (1964).

Prudomestin, isolated from the heartwood of *Prunus domestica*, was given the constitution II based on spectral data, degradation reactions and comparison of the partial ethyl ether (IX) with a synthetic sample.[2] The synthesis of prudomestin itself could not be achieved since the partial demethylation at the 5-position of the main intermediate 3,7-dihydroxy-5,8,4′-trimethoxyflavone (X) was unsuccessful.[2]

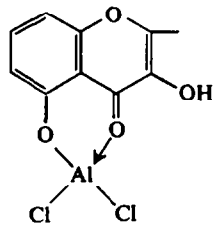


IX R = H; $R_1 = R_2 = C_2H_5$
X R = CH_3; $R_1 = R_2 = H$

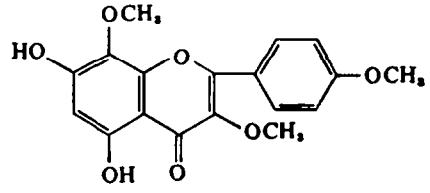
The resistance to selective demethylation under mild conditions of the 5-methoxyl group in the flavonol (X) has been explained on the basis of the ease of formation of the complex XI involving only a proton detachment in preference to the complex XII which would require detachment of the methyl group.[2] An analogous explanation also holds good for the failures experienced in this laboratory to selectively demethylate the 3-methoxyl group of 3-methylprudomestin[7] (XIII). More drastic methods of demethylation affected the 8-methoxyl group also.



XI



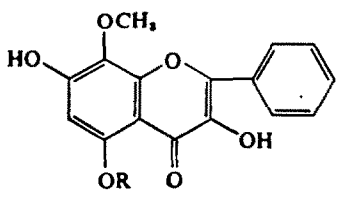
XII



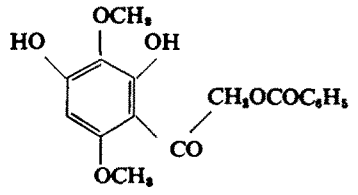
XIII

Since a free hydroxyl at the 3-position hinders selective demethylation of the 5-methoxyl it was decided to block the 3-hydroxyl by formation of an acetate or benzoate. In an exploratory experiment, the synthesis of 3,5,7-trihydroxy-8-methoxyflavone (XIV), a lower analogue of prudomestin, was attempted. 3,7-Dihydroxy-5,8-dimethoxyflavone (XV) was obtained by the Allan-Robinson condensation of ω-benzoyloxy-2,4-dihydroxy-3,6-dimethoxyacetophenone[2] (XVI) with benzoic anhydride. For the demethylation experiments the diacetate and the dibenzoate of the flavonol (XV) were employed. Aluminium chloride in ether at 0° and 35° or in acetonitrile at 100° was used as the demethylating agent. There was easy deacylation with the diacetate whereas with the benzoate at 0° and 35° the compound was recovered unchanged and at 100° only debenzoylation occurred. Thus no selective demethylation could be achieved.

[7] S. C. Bhrara, A. C. Jain and T. R. Seshadri, *Indian J. Chem.* 3, 68 (1965).

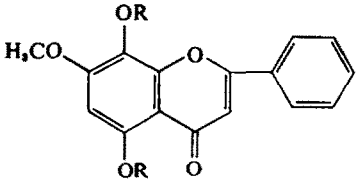


XIV R = H
XV R = CH_3

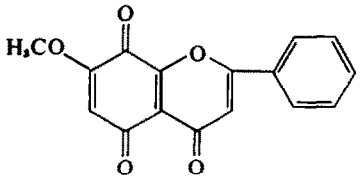


XVI

The above failures prompted a thorough investigation of the various demethylation techniques and eventually the method of oxidative demethylation using nitric acid was chosen. This has been employed earlier for the synthesis of isowogonin[8] (XIX) from 5,7,8-trimethoxyflavone (XVII), with flavoquinone (XVIII) as intermediate. Several other applications of this method are known.[9]

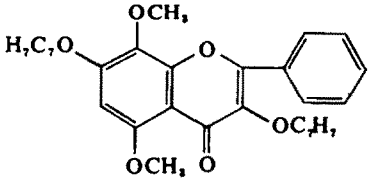


XVII R = CH_3
XIX R = H

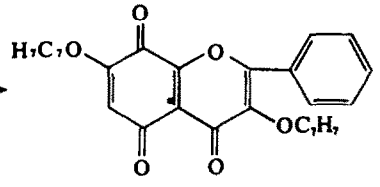


XVIII

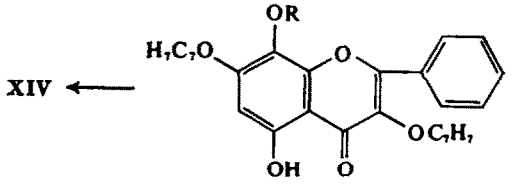
The free hydroxyl groups of XV were protected through benzylation. The dibenzyl ether (XX) on treatment with nitric acid smoothly underwent oxidative demethylation to the flavoquinone (XXI) which exhibited properties characteristic for such compounds.[8,9] It was reduced to the 5,8-dihydroxy compound (XXII) which on partial



XX



XXI



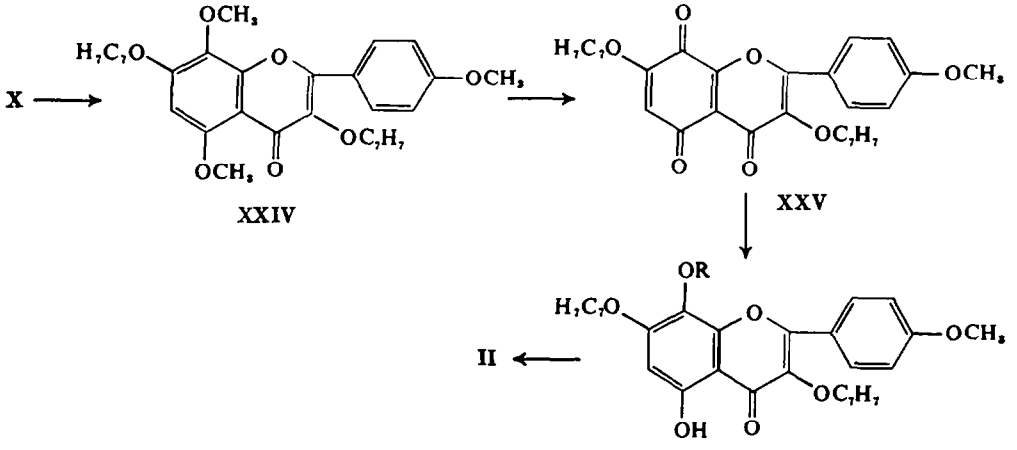
XIV

XXII R = H
XXIII R = CH_3

[8] K. Venkateswara Rao, K. Visveswara Rao and T. R. Seshadri, *Proc. Ind. Acad. Sci.* **25A**, 427 (1947).
[9] K. Visweswara Rao and T. R. Seshadri, *Proc. Ind. Acad. Sci.* **25A**, 397, 417 (1947); K. J. Balakrishna and T. R. Seshadri, *Ibid.* **27A**, 91 (1948).

methylation (XXIII) followed by debenzylation gave a compound which exhibited colour reactions and spectral properties expected of the structure, 3,5,7-trihydroxy-8-methoxyflavone (XIV). The synthesis of the 8-methoxy-5-hydroxy compound was thus achieved in an indirect manner.

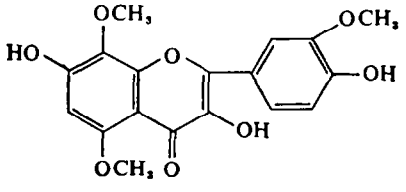
The synthesis of prudomestin itself has now been achieved starting from 3,7-dihydroxy-5,8-4′-trimethoxyflavone[2] (X). The stages in the synthesis are indicated by the formulae XXIV to XXVII. The synthetic sample was found to be identical with the natural one in all respects.



XXIV XXV

XXVI R = H
XXVII R = CH_3

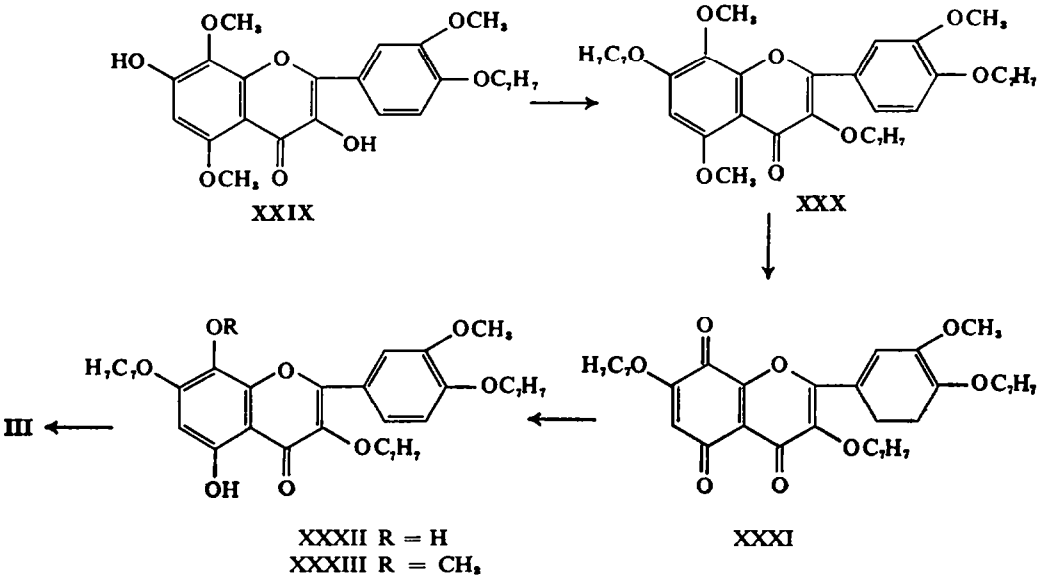
Limocitrin, isolated from the lemon peel by Horowitz and Gentili[3] was given the constitution III based on its conversion to gossypetin hexamethyl ether, the study of the alkali fission products, spectral data and comparison of the 5-methyl ether (XXVIII) with a synthetic sample. The above authors, in an attempt to synthesize limocitrin itself, tried partial demethylation of the 5-methoxyl group of XXVIII but they were unsuccessful.[3]



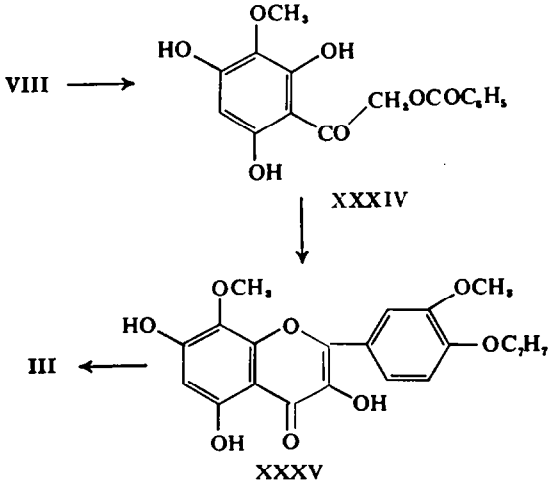
XXVIII

Limocitrin as a higher analogue of prudomestin, it was felt, could be synthesized by an extension of the method employed for the syntheses of XIV and prudomestin (II). The main intermediate was 3,7-dihydroxy-5,8,3′-trimethoxy-4′-benzyloxyflavone (XXIX) obtained by the condensation of XVI with benzylvanillic anhydride and potassium salt of benzylvanillic acid. In this method no aurone was obtained as a by-product.[3] The free hydroxyl groups of XXIX were protected through benzylation and the tribenzyl ether (XXX) was converted into limocitrin (III) passing through the

stages of the flavoquinone (XXXI), the quinol (XXXII) and the 8-0-methyl ether (XXXIII). The synthetic sample was found to be identical in all respects with the natural one.



When the above synthesis was in its last stages Dreyer *et al.*[6] published a synthesis of limocitrin starting from iretol (VIII). This was converted into the ketone (XXXIV) by a Hoesch condensation with benzoyloxyacetonitrile. The ketone (XXXIV) when subjected to the Allan-Robinson reaction using the anhydride of benzylvanillic acid, gave the flavone (XXXV) which was debenzylated to give a compound identical with limocitrin. In this synthesis the cyclization of the unsymmetrical ketone (XXXIV) has been considered to give the 8-methoxy derivative (XXXV). It should however be emphasized that cyclizations involving unsymmetrical ketones are known to give either



the 6-substituted compound alone[10] or a mixture of the 6- and 8-isomers.[11] Hence though Dreyer *et al.* obtained limocitrin, the method is not free from ambiguity. The present method involving the technique of oxidative demethylation is on the other hand unambiguous.

EXPERIMENTAL

The R_f data are for circular paper chromatography. The solvent systems used are: 50% acetic acid (solvent I) and the lower phase of butanol–acetic acid–water (4:1:5 v/v) (solvent II).

3,7-*Dihydroxy*-5,8-*dimethoxyflavone* (XV). A mixture of ω-benzoyloxy-2,4-dihydroxy-3,6-dimethoxyacetophenone[9] (3 g), benzoic anhydride (8·4 g) and sodium benzoate (2·4 g) was heated at 160–170° for 4 hr under diminished press; EtOH (150 ml) and an aqueous solution of NaOH (18 g in 22 ml) were added and the mixture refluxed for 0·5 hr. The solvent was removed *in vacuo*, water added and the solution saturated with CO_2. The precipitated flavone was filtered, washed with water and dried. Crystallization from acetone gave yellow plates (0·8 g), m.p. 256°. (Found: C, 65·4; H, 4·8. $C_{17}H_{14}O_6$ requires: C, 65·0; H, 4·5%.) λ_{max}^{EtOH} ~254, 272, 284 mμ; $\lambda_{max}^{EtOH-NaOAc}$ 282 mμ; $\lambda_{max}^{EtOH-AlCl_3}$ 272, 415 mμ. It gave a greenish brown $FeCl_3$ test.

3,7-*Dibenzyloxy*-5,8-*dimethoxyflavone* (XX). A mixture of XV (0·7 g), freshly distilled benzyl bromide (0·7 ml), anhydrous K_2CO_3 (3 g) and dry acetone (500 ml) was refluxed for about 12 hr till a test portion of the solution gave no colour with ethanolic $FeCl_3$. The potassium salts were filtered off, washed with a little acetone and from the filtrate acetone was removed. Water was added to the residue and excess benzyl bromide removed by steam distillation. The residue was extracted with ethyl acetate, the extract dried and the solvent removed. Crystallization of the yellowish brown semi-solid from acetone yielded yellow needles (0·47 g), m.p. 161–162°. (Found: C, 75·0; H, 5·5. $C_{31}H_{26}O_6$ requires: C, 75·3; H, 5·3%.) λ_{max}^{EtOH} 267, 340 mμ.

3,7-*Dibenzyloxyflavoquinone* (XXI). Compound XX (0·2 g) was treated with HNO_3 (5 ml; *d.* 1·25). The solid immediately acquired a brownish-red colour. The suspension was stirred vigorously with a glass rod for about 20 min with intermittent immersion in a water bath kept at 40°. During this time the solid changed from brownish-red to red and finally to a brick red semi-solid mass and the solution acquired a faint yellow colour. It was kept in an ice-bath for about 30 min during which time the semi-solid mass set to a solid. The solid was filtered off, washed with HNO_3 (*d.* 1·25), water and dried. It was crystallized successively from EtOH, glacial acetic acid and chloroform when it finally came out as brick red plates (0·1 g), m.p. 192–193° (sintering at 170°). Repeated purification did not yield an analytically pure sample; but it was pure enough for the preparation of later stages.

3,7-*Dibenzyloxy*-5,8-*dihydroxyflavone* (XXII). Compound XXI (0·10 g) was dissolved in glacial acetic acid (1 ml) and treated with Na_2SO_3 (0·5 g). The deep red solution became bright yellow. It was heated in a boiling water bath for 1 min, diluted with water and kept aside for 2 hr. The yellow precipitate was then filtered, washed with water and dried. It crystallized from EtOH as yellow clusters (0·06 g), m.p. 142–143°. (Found: C, 74·7; H, 4·9. $C_{29}H_{22}O_6$ requires: C, 74·7; H, 4·8%.) λ_{max}^{EtOH} 279 (4·49), 369 (3·68) mμ. The dihydroxy compound answered the "gossypetone" test[12] and gave a transient green colour changing to brown with ethanolic $FeCl_3$.

3,7-*Dibenzyloxy*-5-*hydroxy*-8-*methoxyflavone* (XXIII). A mixture of XXII (0·065 g), freshly distilled dimethyl sulphate (0·015 ml), anhydrous K_2CO_3 (0·1 g) and dry acetone (50 ml) was refluxed for 10 hr and worked up as usual. The 8-methyl ether crystallized from EtOH as yellowish brown needles (0·040 g), m.p. 177°. (Found: C, 74·7; H, 5·2. $C_{30}H_{24}O_6$ requires: C, 75·0; H, 5·0%.) It gave a green $FeCl_3$ test.

3,5,7-*Trihydroxy*-8-*methoxyflavone* (XIV). A mixture of XXIII (0·03 g), Pd–C (0·05 g; 5%) and ethyl acetate (50 ml) was shaken in the presence of H_2 till two moles of the gas were absorbed. The

[10] W. Baker, *J. Chem. Soc.* 74 (1929); W. Baker and R. Robinson, *Ibid.* 152 (1929); R. L. Shriner and R. W. Stephenson, *J. Amer. Chem. Soc.* **64**, 2737 (1942).

[11] W. Baker, D. F. Downing, A. J. Floyd, B. Gilbert, W. D. Ollis and R. C. Russell, *Tetrahedron Letters* No. 5, 6 (1960); M. Chadenson, J. Chopin and M. Bouillant, *Bull. Soc. Chem. Fr.* 1457 (1962); L. Farkas and J. Varady, *Acta Chim. Acad. Sci., Hung.* **38**, 283 (1963); A. C. Jain and T. R. Seshadri, *J. Sci. Ind. Res.* **12B**, 564 (1953).

[12] A. G. Perkin, *J. Chem. Soc.* 650 (1913).

contents were filtered and the catalyst washed with warm ethyl acetate. From the filtrate ethyl acetate was removed by distillation. The yellow residue crystallized from ethyl acetate as needles (0·01 g), m.p. 221–222°. (Found: C, 64·5; H, 4·1. $C_{16}H_{12}O_6$ requires: C, 64·0; H, 4·0%), λ_{max}^{EtOH} ~ 254 (3·88), 276 (4·17), 375 (3·76) mμ; $\lambda_{max}^{EtOH-NaOAc}$ ~ 271 (3·79), 283 (3·70), 298 (3·97) mμ; $\lambda_{max}^{EtOH-AlCl_3}$ 430 (3·88) mμ, R_f values at 37°: 0·85 (solvent I) and 0·61 (solvent II).

3,7-*Dibenzyloxy*-5,8,4′-*trimethoxyflavone* (XXIV). 3,7-Dihydroxy-5,8,4′-trimethoxyflavone[8] (0·1 g) was dissolved in dry acetone (50 ml) and freshly distilled benzyl bromide (0·1 ml) and anhydrous K_2CO_3 (0·5 g) were added. The mixture was refluxed for 16 hr and worked up as indicated under XX. The trimethoxyflavone crystallized from acetone as yellow needles (0·12 g), m.p. 170°. (Found: C, 72·7; H, 5·7. $C_{32}H_{28}O_7$ requires: C, 73·3; H, 5·4%), λ_{max}^{EtOH} 268, 340 mμ.

3,7-*Dibenzyloxy*-4′-*methoxyflavoquinone* (XXV). The flavone XXIV (0·08 g) was treated with HNO_3 (5 ml; *d.* 1·25) and the reaction carried out as described under XXI. The flavoquinone crystallized from glacial acetic acid as red plates (0·04 g), m.p. 215–217° (sintering at 208°). Repeated purification did not yield an analytically pure sample; but it was pure enough for the next stage.

3,7-*Dibenzyloxy*-5,8-*dihydroxy*-4′-*methoxyflavone* (XXVI). The above XXV (0·06 g) was dissolved in glacial acetic acid (0·5 ml) and treated with Na_2SO_3 (0·3 g). The mixture was heated on a boiling water bath for 2 min and diluted with water; the precipitated quinol was filtered, washed with water, dried and crystallized from EtOH as clusters (0·04 g), m.p. 191–192°. (Found: C, 73·0; H, 4·9. $C_{30}H_{24}O_7$ requires: C, 72·6; H, 4·8%), λ_{max}^{EtOH} 280 (4·37), ~300 (4·19), 369 (3·84) mμ.

3,7-*Dibenzyloxy*-5-*hydroxy*-8,4′-*dimethoxyflavone* (XXVII). A mixture of XXVI (0·04 g), freshly distilled dimethyl sulphate (0·01 ml), anhydrous K_2CO_3 (0·3 g) and acetone (50 ml) was refluxed for 10 hr and worked up as usual. The 8-methyl ether crystallized from EtOH as pale yellow needles (0·03 g), m.p. 159–161°. (Found: C, 72·3; H, 5·4. $C_{31}H_{26}O_7$ requires: C, 72·9; H, 5·1%.) It gave a green $FeCl_3$ test.

3,5,7-*Trihydroxy*-8,4′-*dimethoxyflavone* (*prudomestin*). A mixture of XXVII (0·03 g), Pd–C (0·04 g; 5%) and ethyl acetate (50 ml) was stirred in the presence of H_2, till the theoretical quantity was absorbed. The product crystallized from ethyl acetate as yellow needles, m.p. 207–208° undepressed by admixture with a natural sample.[8] The UV and IR spectra of synthetic and natural samples were identical; λ_{max}^{EtOH} 274 (4·35), 321 (4·13), 375 (4·21) mμ. ν_{max}^{KBr} 3400, 1656 cm^{-1}; R_f values at 30°: 0·68 (solvent I) and 0·39 (solvent II).

3,7-*Dihydroxy*-5,8,3′-*trimethoxy*-4′-*benzyloxyflavone* (XXIX). A mixture of ω-benzoyloxy-2,4-dihydroxy-3,6-dimethoxyacetophenone[8] (1 g), 0-benzylvanillic anhydride (6 g) and potassium salt of 0-benzylvanillic acid (2 g) was heated at 160–170° for 6 hr *in vacuo*. EtOH (120 ml) and NaOHaq (6 g in 25 ml) were then added and the mixture refluxed for 30 min. The solvent was removed *in vacuo*, water added and the solution saturated with CO_2. The precipitated flavone was filtered, washed with water and dried. It crystallized from ethyl acetate as yellowish orange crystals (0·12 g), m.p. 239–241° (lit.[8] 238–241°), λ_{max}^{EtOH} 253, ~272 and 368 mμ. It gave a brown colour with $FeCl_3$.

3,7,4′-*Tribenzyloxy*-5,8,3′-*trimethoxyflavone* (XXX). A mixture of XXIX (0·12 g), freshly distilled benzyl bromide (0·07 ml), anhydrous K_2CO_3 (1 g) and acetone (200 ml) was refluxed for about 16 hr till a test portion gave no colour with ethanolic $FeCl_3$. It was worked up as described under XX. The tribenzyl ether crystallized from ethyl acetate as yellow needles (0·1 g), m.p. 165–166°. (Found: C, 74·6; H, 5·5. $C_{39}H_{34}O_8$ requires C, 74·3; H, 5·4%.)

3,7,4′-*Tribenzyloxy*-3′-*methoxyflavoquinone* (XXXI). The above XXX (0·1 g) was treated with HNO_3 (5 ml; *d.* 1·25) and the reaction carried out as described in the earlier cases. The product crystallized from ethyl acetate as red plates (0·05 g), m.p. 155–157°. An analytically pure sample could not be obtained even after repeated crystallization; but it was pure enough for use in the next stage.

3,7,4′-*Tribenzyloxy*-5,8-*dihydroxy*-3′-*methoxyflavone* (XXXII). The XXXI (0·08 g) was dissolved in glacial acetic acid (1 ml) and the reduction carried out as in the earlier cases using Na_2SO_3 (0·4 g). The quinol crystallized from EtOH as yellow needles (0·05 g), m.p. 174°. (Found: C, 73·1; H, 5·5. $C_{37}H_{30}O_8$ requires: C, 73·7; H, 5·0%.) It exhibited characteristic colour reactions similar to other quinols.

3,7,4′-*Tribenzyloxy*-5-*hydroxy*-8,3′-*dimethoxyflavone* (XXXIII). A mixture of XXXII (0·04 g), distilled dimethyl sulphate (0·008 ml), anhydrous K_2CO_3 (0·2 g) and dry acetone (30 ml) was refluxed for 12 hr and the product worked up as usual. The 8-methyl ether crystallized from MeOH as pale yellow crystals (0·03 g), m.p. 154–156°. (Found: C, 74·0; H, 5·6. $C_{38}H_{32}O_8$ requires: C, 74·0; H, 5·2%), λ_{max}^{MeOH} 255, ~271, 354 mμ. It gave a green $FeCl_3$ test.

3,5,7,4'-*Tetrahydroxy*-8,3'-*dimethoxyflavone* (*limocitrin*). A solution of XXXIII (0·03 g) in ethyl acetate (30 ml) was catalytically debenzylated using Pd–C (0·05 g; 5%). The product crystallized from ethyl acetate as yellow plates, m.p. and mixed m.p. with a natural sample of limocitrin, 271–272°. The UV and IR spectra of synthetic and natural samples were identical; λ_{max}^{EtOH} 258 (4·31), ~274 (4·26), ~335 (4·10), 380 (4·29); ν_{max}^{KBr} 3450, 1660 cm^{-1}. R_f value at 37°: 0·49 (solvent I).

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