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FLAVONOIDS OF CITRUS—VIII

SYNTHESIS OF LIMOCITROL, LIMOCITRIN AND SPINACETIN

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Abstract—Allan-Robinson syntheses of limocitrol (3,5,7,4′-tetrahydroxy-6,8,3′-trimethoxyflavone), limocitrin (3,5,7,4′-tetrahydroxy-8,3′-dimethoxyflavone) and spinacetin (3,5,7,4′-tetrahydroxy-6,3′-dimethoxyflavone) are described. The direction of ring closure to give limocitrin or spinacetin is determined by the blocking group (benzyl or benzoyl) at the 4-position of the B-ring component.

THE genus *Citrus* (Rutaceae) is noteworthy for the large number of highly substituted flavonoids present in the peel of the fruit. Earlier communications from this laboratory reported the isolation of limocitrin from the enzymatic hydrolysates of extracts of lemon peel (*Citrus limon.*)[1,2] This coloring matter was assigned the structure 3,5,7,4′-tetrahydroxy-8,3′-dimethoxyflavone (XV) on the basis of spectral data and the synthesis of its 5-methyl ether.[2] A second flavonol, limocitrol, which was obtained from the same source, was shown by spectral and limited chemical studies to be 3,5,7,4′-tetrahydroxy-6,8,3′-trimethoxyflavone (XI).[3,4] In this paper a synthesis of these pigments is described. In addition, it is shown that a modification of the synthetic route to limocitrin yields an isomer, spinacetin (3,5,7,4′-tetrahydroxy-6,3′-dimethoxyflavone) (XVI), which was isolated from spinach by Zane and Wender[5] while this work was in progress.

The synthetic approach to limocitrol is illustrated in Fig. 1. Resorcinol dimethyl ether (I) when nitrated under mild conditions gave II; under more vigorous conditions a third nitro group was introduced to give V. Compound V was identical with that obtained by methylation of styphnic acid (IV) with diazomethane. Compound II showed aromatic bands at δ 6·66 and 8·70 ppm, which are assigned to the 2- and 5-positions, respectively, on the basis of their chemical shifts. Since these bands are not coupled a symmetrical arrangement of the substituents is proved. Reduction of V with hydrogen and Pd-C under conditions that gave good results with trinitroanisole[6] gave only a mononitrodiaminoresorcinol dimethyl ether. A single methoxy band in the NMR spectrum suggested that this was a symmetrical product, 2-nitro-4,6-diaminoresorcinol dimethyl ether (VI). The desired triamine (VII) was finally obtained by reduction of V or VI at a slightly elevated pressure and temperature. In

* A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

[1] R. M. Horowitz, *J. Amer. Chem. Soc.* **79**, 6561 (1957).
[2] R. M. Horowitz and B. Gentili, *J. Org. Chem.* **26**, 2899 (1961).
[3] R. M. Horowitz and B. Gentili, *J. Org. Chem.* **25**, 2183 (1960).
[4] R. M. Horowitz and B. Gentili, *Tetrahedron* **20**, 2313 (1964).
[5] A. Zane and S. H. Wender, *J. Org. Chem.* **26**, 4718 (1961).
[6] R. E. Damschroder and R. L. Shriner, *J. Amer. Chem. Soc.* **59**, 931 (1937).

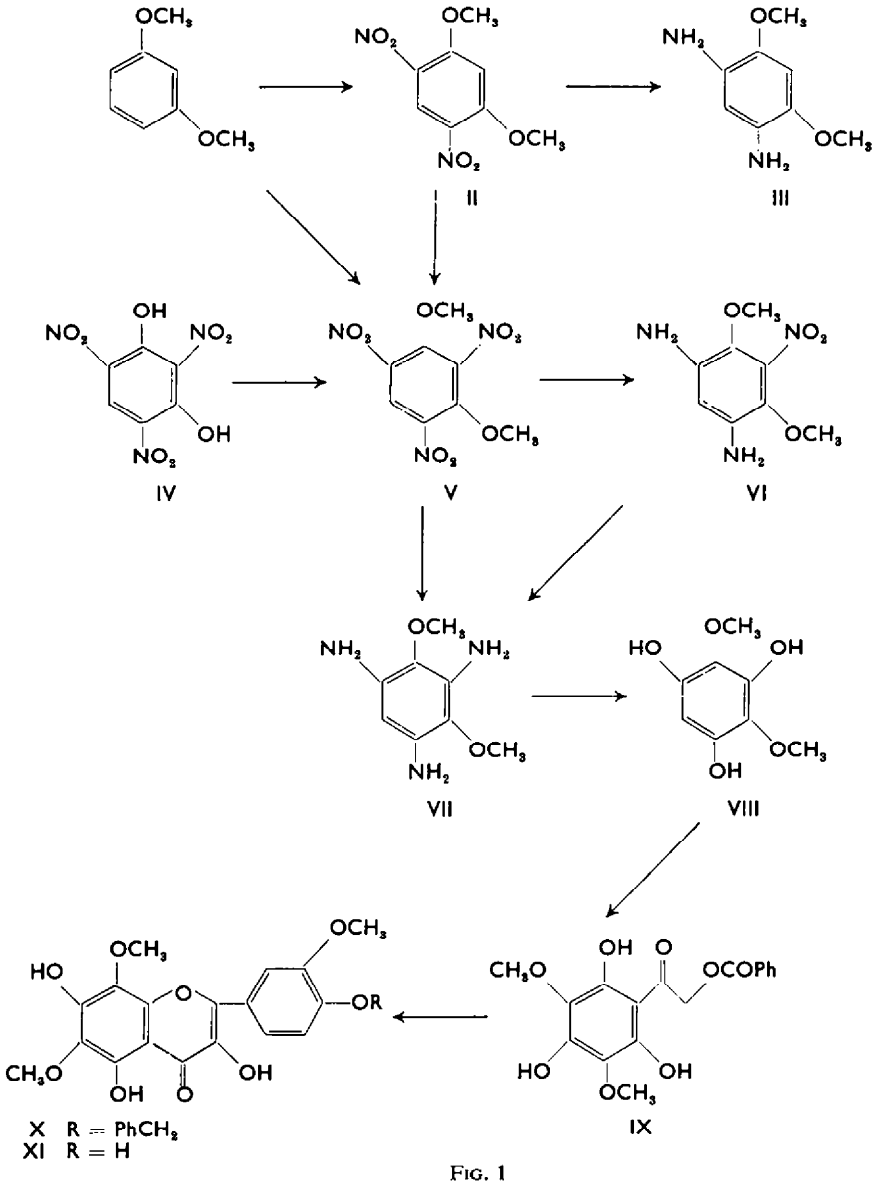


FIG. 1

hydrolyzing VII to dimethoxyphloroglucinol (VIII) it was again necessary to use more vigorous conditions than those required to obtain iretol (XII).[6,7]

Dimethoxyphloroglucinol (VIII) was obtained as a pure crystalline solid by sublimation, but was especially sensitive to decomposition when wet or impure. The

[7] In spite of the higher temps necessary in this case, 2,4,6-triamino-*m*-xylene has been reported[8] to undergo hydrolysis normally at 100°. This suggests that higher temps required in the dimethoxytriamine (VII) case are not due to steric hindrance, but perhaps intramolecular hydrogen bonding between the amino and methoxy groups.

[8] H. Brunmayer, *Monatsh.* **19**, 237 (1898); W. F. Beach, PhD Thesis, California Institute of Technology (1962).

high field position of the aromatic proton of VIII at δ 6·12 ppm testifies to the large concentration of electron donating groups present on the ring. The Hoesch reaction of VIII with benzoyloxyacetonitrile gave the phloracetophenone (IX),[9] which, when condensed with the anhydride of O-benzylvanillic acid *via* the Allan-Robinson reaction, led to the 4′-benzyl ether of limocitrol (X). Removal of the benzyl group by hydrogenation or, more conveniently, acid hydrolysis gave 3,5,7,4′-tetrahydroxy-6,8,3′-trimethoxyflavone (XI), which proved to be identical with the natural limocitrol in all respects.

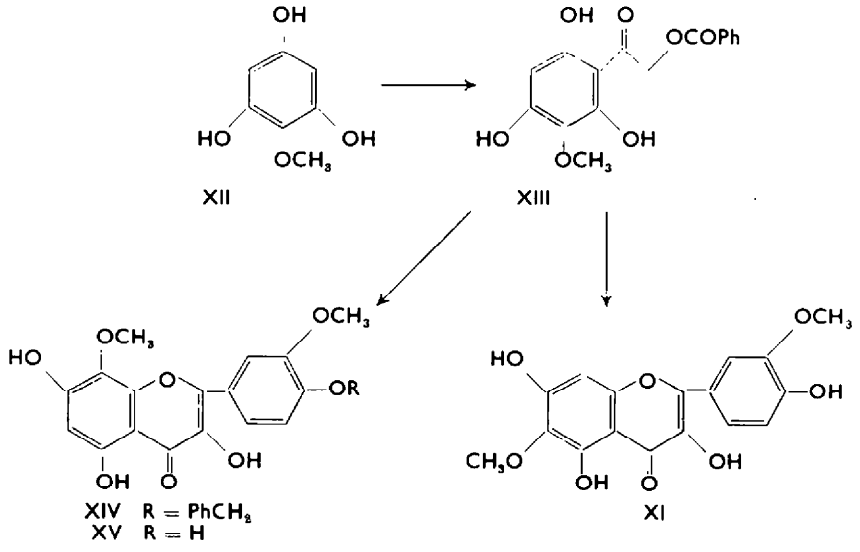


FIG. 2

As seen in Fig. 2, the synthetic route to limocitrin (XV) paralleled that to limocitrol. The unsymmetrical intermediate XIII can lead either to limocitrin (XV) or to spinacetin (XVI).

When the ketone (XIII) was subjected to the Allan-Robinson reaction using the anyhydride of O-benzylvanillic acid, limocitrin 4′-benzyl ether (XIV) was obtained.[11] Heating the benzyl ether (XIV) with acetic–hydrochloric acids converted it to limocitrin (XV), which proved to be identical with the natural pigment in all respects. When the Allan-Robinson reaction was run using the anhydride of O-benzoylvanillic acid, a flavonol different from limocitrin was obtained in very low yield. The spectral properties of the new pigment agreed with those reported for spinacetin (XVI) and were consistent with the structure proposed for that compound.[5] Comparison of an authentic sample obtained through the courtesy of Dr. S. H. Wender proved its identity with the synthetic material. The low yields of spinacetin obtained in this reaction are probably due to the creation of a 3,4′-dihydroxy system during the

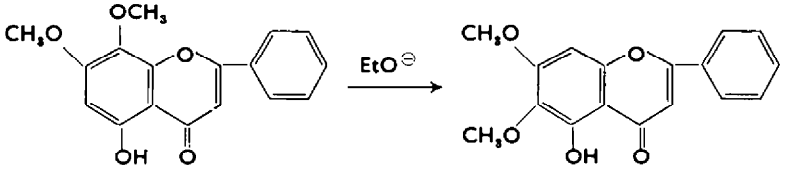
[9] The UV spectrum of this ketone (IX) was very similar to that of 2,6-dihydroxy-3,4,5-trimethoxyacetophenone.[10]

[10] G. H. Stout and V. F. Stout, *Tetrahedron* **14**, 296 (1961).

[11] No evidence for the presence of the 6-methoxy isomer was obtained from an examination of the mother liquors by paper chromatography using 50% acetic acid; however, there is no assurance that paper chromatography could have separated the 6- and 8-isomers had they both been present.

hydrolysis step. This arrangement of free hydroxy groups in flavones is known to be sensitive to base.[12,13]

Orientation problems have been encountered previously in the Allan-Robinson reaction.[14] The factors favoring one orientation over another are not well understood in spite of increased interest in this phase of flavone synthesis in recent years. The lack of detailed knowledge of the mechanism of the reaction precludes assessment of the various factors governing the composition of the products in cases involving unsymmetrical starting ketones. In general, the limited evidence seems to indicate that, in common with the products from the Wessely-Moser rearrangement,[15,16] the products often are those in which maximum hydrogen bonding can occur.[15] The orientation problems under basic conditions have been more extensively studied with isoflavones and flavanones.[17] These are the conditions that lead to extensive decomposition in the case of 3,4'-dihydroxyflavones.[12]



XVII XVIII

In many examples each of the two possible products from an unsymmetrical ketone has been reported from the same reaction.[18] In the present case no evidence for a second isomer could be obtained either preparatively or from an examination of the mother liquors by paper chromatography. A striking example of a base catalyzed rearrangement is the recently reported[19] conversion of 7-methylwogonin (XVII) to 7-methyloroxylin A (XVIII) by ethoxide. The rearrangement here of an 8-substituent to the 6-position suggests that spinacetin (XVI) could also be formed by base through a limocitrin intermediate.

EXPERIMENTAL

The NMR spectra were taken in deuteriochloroform unless otherwise specified. Resonances are given in δ values from internal tetramethylsilane reference. Numbers in parentheses represent

[12] E. B. Dechene, *J. Amer. Pharm. Assoc.* **40**, 495 (1951); L. Jurd and R. M. Horowitz, *J. Org. Chem.* **22**, 1618 (1957).

[13] L. Jurd, *The Chemistry of Flavonoid Compounds* (Edited by T. A. Geissman) pp. 126–127, Macmillan New York (1962).

[14] F. Wessely and G. H. Moser, *Monatsh.* **56**, 97 (1930); R. C. Shah, C. R. Mehta and T. S. Wheeler, *J. Chem. Soc.* 1555 (1938).

[15] T. R. Seshadri, *The Chemistry of Flavonoid Compounds* (Edited by T. A. Geissman) pp. 182–189. Macmillan, New York (1962).

[16] T. S. Wheeler, *Rec. Chem. Progress* **18**, 133 (1957).

[17] See for example: W. Baker, D. F. Downing, A. J. Floyd, B. Gilbert, W. D. Ollis and R. C. Russell, *Tetrahedron Letters* No. 5, 6 (1960); W. Rahman and K. T. Nasim, *J. Org. Chem.* **27**, 944, 4215 (1962); Y. Kawase, Y. Fujino, Y. Ichioka and K. Fukui, *Bull. Chem. Soc., Japan* **30**, 689 (1957); A. Ghanim, A. Zaman and A. R. Kidwai, *Tetrahedron Letters* 185 (1964); M. O. Farooq, W. Rahman and K. T. Nasim, *J. Org. Chem.* **27**, 944 (1962).

[18] See, for example: M. Chadenson, J. Chopin and M. Bouillant, *Bull. Soc. Chim., Fr.* 1457 (1962); S. F. Dyke, W. D. Ollis and M. Sainsbury, *J. Org. Chem.* **26**, 2453 (1961); L. Farkas and J. Varady, *Acta Chim. Acad. Sci., Hung.* **38**, 283 (1963), *Chem. Abstr.* **59**, 13933 (1963).

[19] L. Farkas, A. Major and J. Strelisky, *Chem. Ber.* **96**, 1684 (1963).

relative areas. R_f data are for a 50% acetic acid solvent system unless specified otherwise. The authors are indebted to L. M. White of the Western Regional Research Laboratory for the analytical data.

4,6-*Dinitroresorcinol dimethyl ether* (II). A solution of 39 g (37 ml) of resorcinol dimethyl ether in 140 ml conc H_2SO_4 was warmed on a steam bath for 5 min or until the mixture set to a white mass. The mixture was cooled in Dry Ice–acetone and 50 ml conc HNO_3 slowly added dropwise with good stirring. It was then warmed on a steam bath for 2 hr with occasional stirring and decomposed with a large volume water. The product was crystallized from ethanol or ethyl acetate-ethanol; m.p. 155·5–158°; lit[20] m.p. 157°; NMR, 6·66 (1H at 2-position), 8·70 (1H at 5-position), 4·10 (6H, methoxy) ppm. (Found: C, 42·3; H, 3·46; CH_3O, 27·2. Calc. for $C_8H_8N_2O_6$: C, 42·11; H, 3·53; CH_3O, 27·1%). This dinitro derivative could be converted to the trinitro derivative by further nitration with conc H_2SO_4 and fuming HNO_3, as described below.

4,6-*Diaminoresorcinol dimethyl ether* (III). 4,6-Dinitroresorcinol dimethyl ether in ethyl acetate–ethanol was reduced with Pd–C in a Parr apparatus at room temp. The material rapidly took up the calculated amount of H_2 and when this ceased, the solution was filtered and the solvent removed *in vacuo*, whereupon the product crystallized; m.p. 154–155·5°, from ethanol; NMR, 6·45 (1H, aromatic), 6·16 (1H, aromatic), 3·77 (6H, methoxy) ppm. (Found: C, 57·2; H, 7.15; N, 16·6. $C_8H_{12}O_2N_2$ requires: C, 57·12; H, 7·19; N, 16·65%).

The diacetyl derivative, prepared by adding acetic anhydride to the diamine and allowing it to stand 45 min at room temp, had m.p. 199–200° after two crystallizations from ethanol. (Found: C, 57·2; H, 6·41; N, 11·2. $C_{12}H_{16}N_2O_4$ requires: C, 57·13; H, 6·39; N, 11·10%).

2,4,6-*Trinitroresorcinol dimethyl ether* (V). The nitration was carried out as described for the dinitro derivative. After the initial reaction, 30–40 ml fuming HNO_3 was added and the mixture heated on a steam bath for 3–4 hr. The mixture was allowed to stand overnight and was then decomposed with a large excess of water. The product, crystallized from ethanol or ethanol–ethyl acetate, had m.p. 123·5–125°; lit.[20] m.p. 124–125°; 60% yield. NMR, 8·70 (1H at 5-position), 4·15 (6H, methoxy) ppm. (Found: C, 35·3; H, 2·58; N, 15·4. Calc. for $C_8H_7N_3O_8$: C, 35·17; H, 2·57; N, 15·4%). This product was also obtained by treating styphnic acid with excess diazomethane.

2-*Nitro*-4,6-*diaminoresorcinol dimethyl ether* (VI). The trinitroresorcinol dimethyl ether was reduced with Pd–C in ethyl acetate in a Parr apparatus at room temp. Initial H_2 uptake was rapid and when complete (about 10 min) the solution was filtered and the solvent removed *in vacuo*. The residue was crystallized from ethanol; m.p. 134–135°; ν 3430, 3325 (NH) cm^{-1} (nujol); NMR, 6·23 (1H, aromatic), 3·80 (6H, methoxy) ppm. (Found: C, 45·2; H, 5·25. $C_8H_{11}N_3O_4$ requires: C, 45·06; H, 5·20%).

Treatment of the diamine with acetic anhydride followed by warming on a steam bath gave the 4,6-diacetyl derivative; m.p. 251–252° from ethanol; ν 3225 (NH), 1666 (carbonyl) cm^{-1} (nujol). (Found: C, 48·4; H, 5·05; N, 14·1. $C_{12}H_{15}N_3O_6$ requires: C, 48·81; H, 5·08; N, 14·17%).

2,4,6-*Triaminoresorcinol dimethyl ether* (VII). The trinitroresorcinol dimethyl ether was reduced with Pd–C in ethyl acetate using a Parr apparatus at 45–50° at about 50 lbs pressure. H_2 uptake required 1·5–2·5 hr. A generous amount of catalyst seemed to speed H_2 uptake slightly and when the required amount was consumed, the solution was filtered from the catalyst and the solvent removed *in vacuo*. The residue crystallized upon adding a small amount of ethanol. Further crystallization from ethanol gave a product with m.p. 109·5–111·5°; yield 70–75%. NMR, 5·58 (1H, aromatic), 3·78 (6H, methoxy) ppm. (Found: C, 52·4; H, 7·09; N, 22·9. $C_8H_{12}N_3O_2$ requires: C, 52·44; H, 7·15; N, 22·9%).

Acetylation of the triamine with acetic anhydride at 100° gave the 2,4,6-triacetyl derivative; double m.p. 223–224° and 252–254° from ethyl acetate–ethanol; ν 3225 (NH), 1660 (carbonyl) cm^{-1} (nujol). (Found: C, 54·2; H, 6·03; N, 13·6. $C_{14}H_{19}N_3O_5$ requires: C, 54·36; H, 6·19; N, 13·58%).

Dimethoxyphloroglucinol (VIII). Hydrolysis of the triamine (VII) under conditions that gave good results in the preparation of iretol[6] were found to be unsatisfactory for the dimethoxy derivative (VII). Many runs were made in an attempt to find the optimum conditions for maximum conversion. The reaction was monitored by paper chromatography using 10% acetic acid as the irrigant; alcoholic vanillin solution followed by HCl gas was used to visualize the spots.

[20] M. Hoenig, *Ber. Dtsch. Chem. Ges.* **11**, 1039 (1878).

A solution of 2 g of the triamine in 100 ml of distilled water containing 2 ml HCl aq and 2 g $SnCl_2$ was sealed in a pressure bottle under N_2. The bottle was heated in an autoclave at 120–122° for 28 hr. After cooling and filtering the solvent was removed *in vacuo*. The residue was extracted with ether until the extracts no longer gave a vanillin-HCl test (ca. 15X). The ether extracts were dried and the ether removed *in vacuo*. The last traces of water were removed at 1–2 mm Hg until a heavy brownish oil or semisolid remained. This material was best used at once in the preparation of the phloracetophenone derivative (IX). When impure, the product quickly darkens upon standing even in the cold, and must be used within a few hr. An analytical sample, prepared by sublimation followed by crystallization from benzene, had m.p. 150°. It gave a brown $FeCl_3$ test. NMR, 6·12 (1H, aromatic), 3·75 (9H, methoxy) ppm. (in D_2O, relative to Tiers' salt[21]). (Found: C, 51·8; H, 5·41; CH_3O, 32·7. $C_8H_{10}O_5$ requires: C. 51·61; H, 5·41; CH_3O, 33·3%).

Acetylation with pyridine-acetic anhydride gave the triacetate, which could not be obtained crystalline. NMR, 6·75 (1H, aromatic), 3·75 (6H, methoxy), 2·32 (3H, acetoxy), 2·25 (6H, acetoxy) ppm.

Benzoyloxyacetonitrile.[22] A solution of 24 g of NaOH in 150 ml water was slowly added with stirring to a solution of 70% glycolonitrile with ice bath cooling such that the temp was kept below 30°. This was followed by the dropwise addition of 86 g benzoyl chloride at such a rate that the temp was kept below 25°. Stirring was continued for 15 min after all the benzoyl chloride had been added. The resulting heavy oil was separated from the aqueous phase, washed with water, dried, and distilled; b.p. 110–120°/1 mm; 80–90% yields.

3,5-*Dimethoxy-ω-Benzoyloxyphloracetophenone* (IX). To the crude, dry VIII from the above reaction dissolved in anhydrous ether, was added 1 g benzoyloxyacetonitrile and 2 g anhydrous $ZnCl_2$. The mixture was saturated with HCl gas with ice bath cooling. After standing overnight in the freezer, a heavy oil separated. Water was added to the oil and the resulting solution heated on a steam bath for 15 min. Upon cooling, an oil separated which was taken up in ether, washed with 5% $NaHCO_3$aq and then with 5% Na_2CO_3. Acidification of the carbonate extracts gave crystalline material if the starting material was especially pure, but more often the product was an oil. The acidified product was taken up in ether, the ether phase dried, solvent removed, and the residue crystallized twice from benzene; m.p. 139–142°; yield 12–15% from the triamine; m.p. 140 142° after sublimation in high vacuum. The product gave a dirty brown $FeCl_3$ test; ν 1749 (ester) 1639–1605 (broad unresolved band) cm^{-1} (nujol); λ_{max}^{EtOH} 228 (26,400) 293(22,600), 345(2,700) mμ, $\lambda_{max}^{EtOH-NaOH}$ 224, 328 mμ; NMR, 5·55 (2H,—$COCH_2O$—), 3·75 (6H, methoxy) ppm. (Found: C, 58·8; H, 4·60; CH_3O, 17·4. $C_{17}H_{16}O_8$ requires: C, 58·62; H, 4·63; CH_3O, 17·81%).

Limocitrol 4′-benzyl ether (X). A mixture of 1 g IX and 6 g O-benzylvanillic anhydride was heated with 2·1 ml freshly distilled triethylamine at 160–190° for 6 hr under N_2.[23] A solution of 6 g NaOH in 80 ml 75% ethanol was then added and the mixture refluxed for 20 min. The solvent was removed *in vacuo*, water added, the solution saturated with CO_2, and extracted with ethyl acetate. The ethyl acetate extracts were dried, solvent removed, and the residue recrystallized from ethyl acetate–methanol to give 175 mg of product; a second crop of 80 mg was obtained; 13·5% yield. An analytical sample gave m.p. 176–178° from ethyl acetate–methanol; R_f 0·60; ν 1639 (carbonyl) cm^{-1} (nujol). The carbonyl band was of relatively low intensity. λ_{max}^{EtOH} 256(21,500), 279(14,500). 349(11,100), 376(12,300) mμ; $\lambda_{max}^{EtOH-NaOH}$ 286, 329, 423 mμ. The other UV shifts were consistent with the assigned structure.[24] (Found: C, 64·6; H, 4·87; CH_3O, 20·0. $C_{25}H_{22}O_9$ requires: C, 64·37; H, 4·75; CH_3O, 20·13%).

The triacetate was prepared with acetic anhydride–sodium acetate; m.p. 177·5–178·5 from ether–methanol. (Found: C, 62·8; H, 5·01. $C_{37}H_{28}O_{12}$ requires: C, 62·8; H, 4·76%).

Isorhamnetin 4′-benzyl ether. When the same reaction was run using 1 g of ω-benzoyloxyphloracetophenone a 125 mg yield of isorhamnetin 4′-benzyl ether was obtained; m.p. 224–228° (methanol) giving a green-brown $FeCl_3$ test. An analytical sample prepared by sublimation gave m.p. 245–247·5°; λ_{max}^{EtOH} 255, ~310, 369 mμ; $\lambda_{max}^{EtOH-NaOH}$ 271, 279, ~320, 416 mμ. The other UV shifts were consistent with the assigned structure.[24] (Found: C, 67·9; H, 4·51. $C_{23}H_{18}O_7$ requires: C, 67·97; H, 4·46%).

Limocitrol (XI). A solution of 11 mg X in 0·5 ml glacial acetic acid and 0·5 ml conc HCl was

[21] G. V. D. Tiers and R. I. Coon, *J. Org. Chem.* **26,** 2097 (1961).

[22] M. Aloy and C. Rabaut, *Bull. soc. chim., Fr.* **13,** 457 (1913).

[23] This is the Allan-Robinson reaction as modified by R. Kuhn and I. Loew: *Ber. Dtsch. Chem. Ges.* **77B,** 196 (1944).

[24] Reference 13, pp. 107–131.

heated for 0·5 hr on a steam bath. This solution was then concentrated and a small amount of methanol added. The product separated upon cooling; m.p. 210–211° from methanol or 221–222° from ethanol, each form having a distinctive IR spectrum in KBr. The synthetic sample gave no depression in m.p. when mixed with an authentic sample[4]. The IR and UV spectra of natural and synthetic samples were identical; ν 1641 (carbonyl) cm^{-1} (KBr); the carbonyl band was again of low intensity; R_f 0·54. Limocitrol was also prepared from the 4′-benzyl ether by hydrogenation in ethyl acetate with Pd/C.

Limocitrol tetraacetate was prepared by acetylation with acetic anhydride–sodium acetate; m.p. 202–204° from methanol. Mixed m.p. gave no depression with an authentic sample. The UV and IR spectra were identical with those obtained from a natural sample;[4] ν 1782 (acetate), 1653 (carbonyl) cm^{-1} (KBr); λ_{max}^{EtOH} 250, 262, 320 mμ. NMR, 4·00, 3·89, 3·87 (1:1:1, methoxy), 2·46, 2·40, 2·33, 2·32 (1:1:1:1, acetoxy) ppm.

3-*Methoxy-ω-benzoyloxyphloracetophenone* (XIII). To 2·13 g iretol[6] was added 2·2 g benzoyloxyacetonitrile, 4 g anhydrous $ZnCl_2$, and 250 ml anhydrous ether. The mixture was cooled in an ice bath and saturated with HCl gas, whereupon a heavy oil separated. The solution was kept at 0° for 22–24 hr. The ether was then decanted from the oil and the oil was warmed on a steam bath with 100 ml water for 30 min, cooled, and the product collected. Recrystallization from methanol–water gave 1·2 g product; m.p. 218–222°. The ketone gave a brown $FeCl_3$ test; ν 1696 (ester), 1664 (ketone), 1619 (aromatic) cm^{-1} (nujol); λ_{max}^{EtOH} 229(21,000), 290(18,500), 332(3,500) mμ; $\lambda_{max}^{EtOH-NaOH}$ 228, 324 mμ; NMR, 6·03 (1H, aromatic) 5·52 (2H, —$COCH_2O$—), 3·73 (3H, methoxy) ppm. A sample was sublimed for analysis, m.p. 227–229°. (Found: C, 60·4; H, 4·53. $C_{16}H_{14}O_7$ requires: C, 60·37; H, 4·43%).

Limocitrin 4′-benzyl ether (XIV). A mixture of 1 g XIII, 6 g O-benzylvanillic anhydride, and 2·3 ml triethylamine was heated at 180–190° for 5·5 hr under N_2. A solution of 5 g NaOH in 60 ml 65% ethanol was added and the solution refluxed an additional 0·5 hr under N_2. The solvent was removed *in vacuo*, 75 ml water added, and the resulting solution saturated with CO_2. The mixture was extracted with ethyl acetate. The ethyl acetate extracts were dried and concentrated, ethanol added and the solution cooled. The product was collected and recrystallized from ethyl acetate–methanol, m.p. 247–249°; dirty green $FeCl_3$ test; R_f 0·62; ν 3395 (hydroxy), 1658 (carbonyl) cm^{-1} (KBr); λ_{max}^{EtOH} 258(18,000), 273(15,000), 335(11,300), 376(16,500) mμ; $\lambda_{max}^{EtOH-NaOH}$ 287, ~313, 428 mμ. (Found: C, 65·6; H, 4·71. $C_{24}H_{20}O_8$ requires: C, 66·05; H, 4·62%).

The triacetate was prepared by acetylation with acetic anhydride–sodium acetate; m.p. 172·5–173·5° from ethanol. (Found: C, 63·5; H, 4·79. $C_{30}H_{26}O_{11}$ requires: C, 64·0; H, 4·65%).

Limocitrin (XV). A suspension of 11 mg XIV in 1 ml acetic acid and 0·5 ml conc HCl was heated on a steam bath for 45 min. The material slowly went into solution. The solution was then filtered while hot, and water slowly added to the filtrates to the cloud point. After cooling, the product was collected; m.p. 269–271° (dec). Sublimation at 0·3 mm/190° failed to raise the m.p. There was no depression in m.p. upon admixture with a natural sample.[2] The UV and IR spectra of synthetic and natural samples were identical; ν 3450(hydroxy), 1657(carbonyl) cm^{-1} (KBr); green-brown $FeCl_3$ test; R_f 0·39, identical with a natural sample; λ_{Max}^{EtOH} 259(10,500), ~273(8,100), ~311(3,900), ~340(5,800), 378(10,000) mμ.

Limocitrin tetraacetate[2] gave the following NMR spectrum: 4·18, 4·07 (1:1, methoxy), 2·52, 2·48, 2·45, 2·43 ((1:1:1:1, acetyl) ppm.

Spinacetin (XVI). A mixture of 1 g 3-methoxy-ω-benzoyloxyphloracetophenone, 6 g O-benzoylvanillic anhydride, and 2·3 ml triethylamine was heated 5·5 hr at 185–200° under N_2. A solution of 5 g NaOH in 50% ethanol was added and the solution refluxed for 25 min. The solvent was removed with a rotary evaporator, the residue dissolved in water and saturated with CO_2. The solution was extracted 4X with ethyl acetate, the combined ethyl acetate extracts dried, and solvent removed. The residue was chromatographed over a short column of silicic acid with chloroform. The chloroform eluants which contained mostly guaiacol were discarded. The pigment was eluted with 15–20% ethyl acetate in chloroform, the solvent removed from the eluants and the residue crystallized from benzene and then benzene–methanol; m.p. 228–232°. The spectra were identical with those of an authentic sample;[5] ν 1660 cm^{-1} (KBr); λ_{Max}^{EtOH} 257, 368 mμ; $\lambda_{Max}^{EtOH-AcONa}$ 269, ~305, 322, 383 mμ; $\lambda_{Max}^{EtOH-AlCl_3}$ 269, 435 mμ; R_f 0·45. The spot was much lighter than that from limocitrin under UV light.

Acknowledgements—The authors are indebted to Mr. Bruno Gentili for helpful technical assistance and to Dr. S. H. Wender for a sample of natural spinacetin.