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THE STRUCTURE OF EXOSTEMIN AND SYNTHESIS OF SOME RELATED 4-PHENYL COUMARINS

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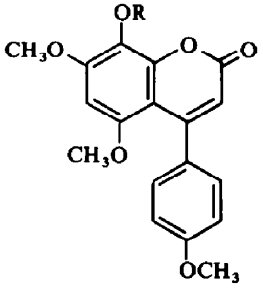
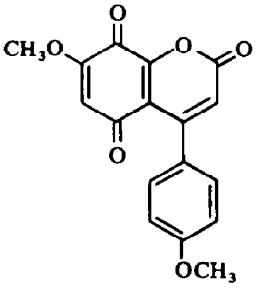
Abstract—8-Hydroxy-5:7-dimethoxy-4-(*p*-methoxyphenyl) coumarin has been synthesized by condensing 1,2-dihydroxy-3,5-dimethoxybenzene with p-methoxybenzoylacetic ester. The m.p. of the synthetic compound and its acetate differ from the reported m.p. of exostemin and its acetate but there is general agreement in spectral data. The simpler 4-phenyl analogue has also been synthesized. The m.ps of the simpler analogue, its methyl ether and acetate are remarkably close to those of exostemin and its derivatives indicating the possibility of some error in the study of the natural product.

EXOSTEMIN (Ia), the principal constituent of the benzene extract of the Mexican plant *Exostemma caribaeum* (family *Rubiacea*; sub family *Cinconidea*) is the second example of a coumarin with a substituted 4-phenyl ring. A tentative structure for this compound was proposed[1] as Ia on the basis of analytical and spectral data of the compound, its methyl ether (Ib) and the quinone (II) formed from it by chromic acid oxidation. The 5:7:8-oxygenation pattern is unusual in the neoflavanoids, moreover this type of partial methylation with a free 8-OH group has not been observed earlier in either the flavonoid or neoflavanoid (C_{15}) series; hence it was necessary to provide synthetic confirmation. In this paper we describe an unambiguous synthesis of Ia and related 4-phenylcoumarins.



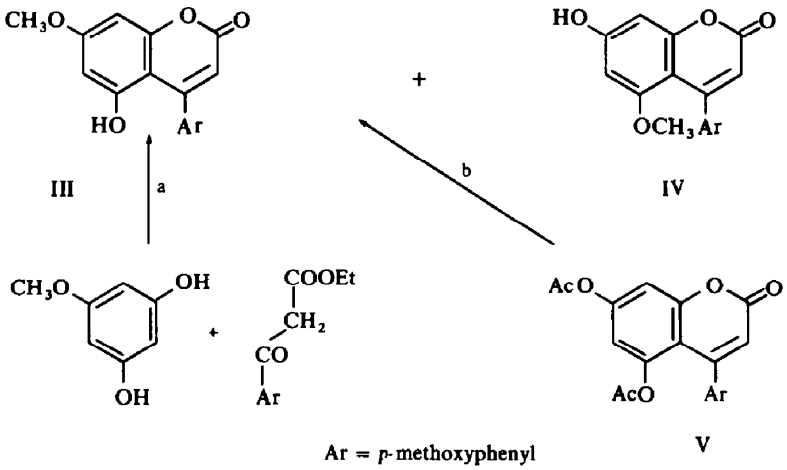
Ia: R = H
b: R = CH_3



II

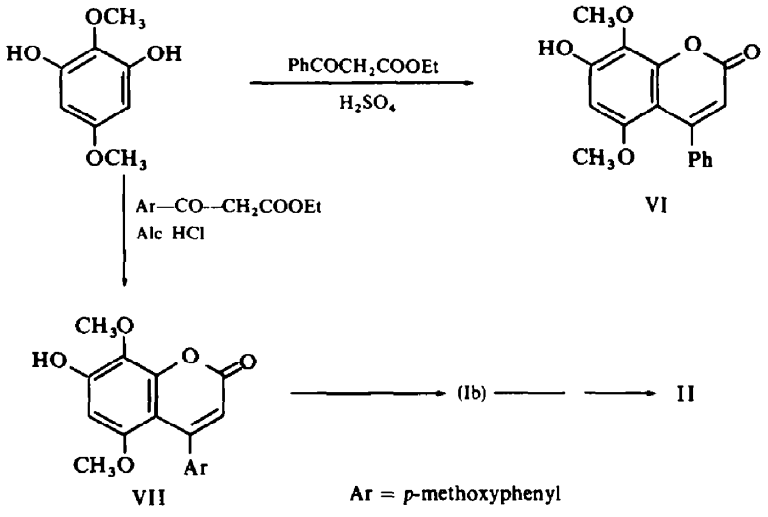
As the first step, the tetramethyl ether (Ib) was synthesized. Nuclear oxidation of 7-methoxy-5-hydroxy-4-(*p*-methoxyphenyl) coumarin (III) appeared to be the simplest route to the final 5:7:8-oxygenation pattern. The required partial methyl ether (III) was obtained in two ways. (a) Pechmann condensation of monomethyl phloroglucinol[2] with *p*-methoxybenzoylacetic ester[3] gave a mixture of almost equal amounts of the isomeric methyl ethers (III and IV); column chromatography on silica gel separated

this mixture. The 7-methyl ether (III) was eluted first and was identical with the product obtained by route (b) given below. The other methyl ether eluted next, should therefore, have the isomeric structure IV. (b) The second method was far better. It consisted of the methylation of 5,7-diacetoxy-4-(*p*-methoxyphenyl) coumarin (V)[4] with methyl iodide and potassium carbonate in refluxing acetone and gave the acetate of the partial methyl ether as the sole product in excellent yield. The feasibility of preparing partial methyl ethers, especially 7-methyl ethers of polyhydroxy coumarins by this method has been established by the recent work of Ahluwalia *et al.*[5] 5,7-Diacetoxy-coumarin itself gives a mixture of monomethyl ethers. In the 4-methyl derivative, the steric effect of the substitutent protects the 5-acetoxyl group and the 7-monomethyl ether is obtained easily. The 4-phenyl substituent in V serves similarly in producing 7-methylation. The nuclear oxidation of this partial methyl ether (III) did not proceed satisfactorily with persulphate as only a poor yield of ill defined products were obtained. Oxidation with nitric acid also gave an intractable mixture of products but TLC showed the presence of the quinone II. The separation of this mixture was not practicable and hence this route was abandoned.



In another route 7-hydroxy-trimethoxycoumarin (VII) was used as intermediate. Although the condensation of 2,6-dihydroxy-1,4-dimethoxybenzene[6] with benzoylacetic ester proceeded well in the presence of conc. sulphuric acid to give VI similar condensation of the phenol with *p*-methoxybenzoylacetic ester gave only poor yields of the *p*-methoxy analogue (VII). The reaction proceeded smoothly, however, when alcoholic hydrogen chloride[7] was used as the condensing agent. The product VII, obtained in good yield, gave the tetramethyl ether (Ib) on methylation with diazomethane. The m.p. of this methyl ether (142–143°) agreed closely with that reported for exostemin methyl ether (144–145°). The IR and UV spectral data of the two also agreed closely. However, the NMR spectrum of the synthetic methyl ether (methoxyl peaks at δ 3·47, 3·86, 3·92 and 3·97 ppm) was somewhat different from the spectrum reported for exostemin methyl ether (OMe peaks at δ 3·43, 3·50, 3·85 and 3·97 ppm). The latter consists of two pairs of signals well separated from each other whereas

the spectrum of Ib obtained now has one signal (δ 3·47 ppm) separate from a group of 3 other signals. This is more consistent with Ib and with the spectrum of the simpler model compound (Table I). As a further check the tetramethyl ether (Ib) was converted into the quinone (II). For this purpose chromic acid was not good; by using nitric acid (*d* 1·2) a quantitative conversion was obtained. The properties of the quinone (m.p., UV spectra) agreed closely with those reported.



Since the NMR data of the synthetic coumarin (Ib) do not agree closely with the reported data of exostemin methyl ether, it was necessary to synthesize 8-hydroxy-5,7-dimethoxy-4-(*p*-methoxyphenyl) coumarin and compare it with exostemin. For this synthesis, it was hoped that the diacetoxycoumarin (VIII) obtained from the quinone (II) by reduction followed by acetylation, would undergo partial methylation at the 5-position when heated with methyl iodide in refluxing acetone. Actually, however the methylation proceeded extremely slowly and gave a mixture of products which contained (TLC) the desired coumarin (Ia) in small amounts but isolation proved difficult. This seems to be due to the existence of competing factors, (1) steric hinderance affecting position 5 and (2) effect of neighbouring ring oxygen affecting position 8. The interaction of ring oxygen with an 8-OH group has earlier been discussed in connection with 8-hydroxyflavanones and related compounds.[8]

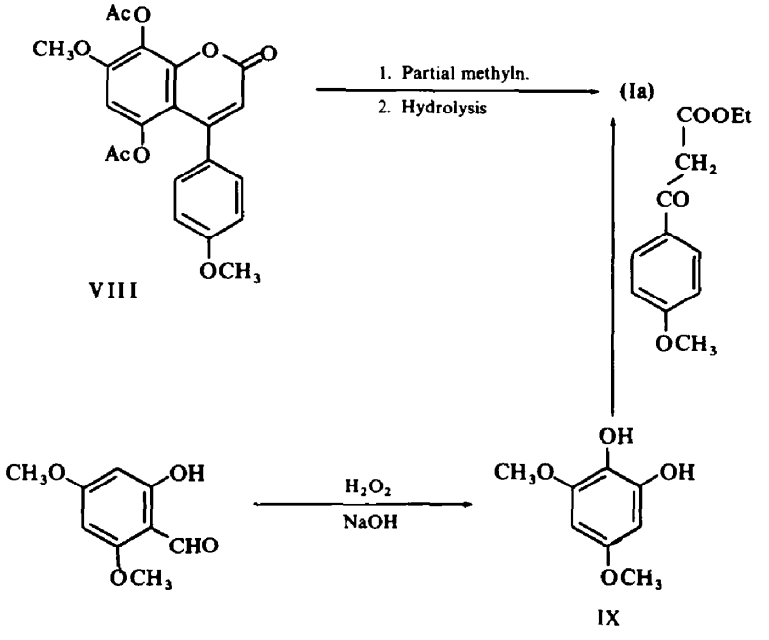
In an alternative route, the condensation of 1,2-dihydroxy-3,5-dimethoxybenzene (IX) or its acetate, obtained by Dakin's oxidation of 2-hydroxy-4,6-dimethoxybenzaldehyde, with *p*-methoxybenzoylacetic ester in alcoholic hydrogen chloride, gave Ia directly in good yield. Although it was a yellow crystalline compound as reported for exostemin, its m.p. was 195–196°, quite different from the reported m.p. 173° for exostemin. The analytical and spectral data, and the properties of the synthetic compound agree well with structure Ia. Moreover, with diazomethane, it gave the tetramethoxycoumarin (Ib), identical with the substance obtained by the earlier route. It also gave the same quinone (II) by oxidation with nitric acid. Thus there is no doubt about the structure Ia of the synthetic compound. The acetate of Ia had a

TABLE 1.* NMR DATA OF COUMARINS STUDIED

Compounds			δ ppm				Aromatic proton of 4-phenyl ring. Centre of the Quatret
			—CO—CH_3	—OCH_3	H_3	H_6	
8-Hydroxy-5,7-dimethoxy-4-(p-methoxyphenyl)	Ia	Synth.	—	3·43, 3·85, 3·97	5·98	6·35	7·06
Exostemin		Nat.	—	3·43, 3·85, 3·97	6·00	6·35	7·06
5,7,8-Trimethoxy-4-(*p*-methoxyphenyl)	Ib	Synth.	—	3·47, 0·00, 3·86, 3·92, 3·97	5·98	6·34	7·05
Exostemin tetramethyl ether		Nat.	—	3·43, 3·50, 3·85, 0·00, 3·97	6·00	6·35	7·06
Acetate of Ia		Synth.	2·35	3·44, 3·78, 3·88	—	—	—
Exostemin acetate		Nat.	2·37	3·43, 0·00, 3·85, 3·97	—	—	—
7-Hydroxy-5,8-dimethoxy-4-(*p*-methoxyphenyl)	VII	Synth.	—	3·42, 3·82, 4·02	5·96	6·37	7·05
5,7,8-Trimethoxy-4-phenyl	XI	Synth.	—	3·35, 3·87, 3·95	5·95	6·27	—

* Spectrum taken in $CDCl_3$ solution with T.M.S. as internal standard in 60 Mc/s Varian instrument.

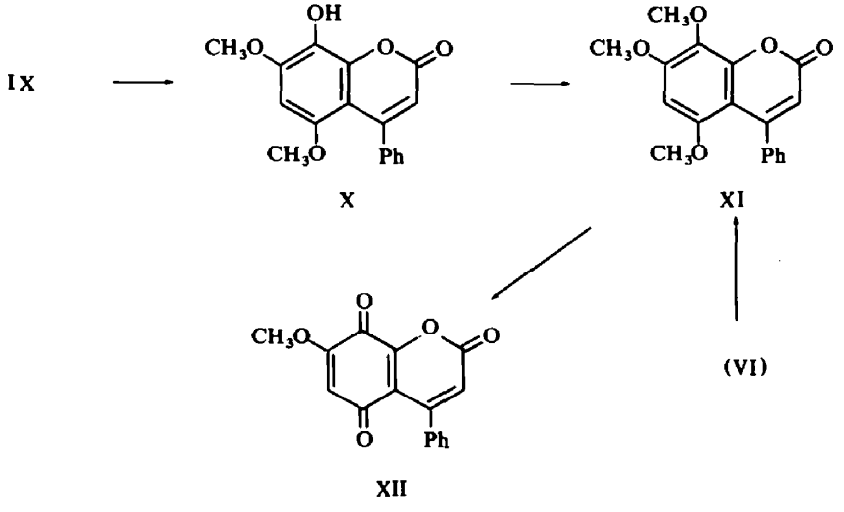
m.p. 187–188°, also different from the reported m.p. 203–204° for exostemin acetate. Although the NMR spectrum of synthetic 8-hydroxy compound (Ia) agrees with that of exostemin itself, the spectrum of acetate of Ia differs from the reported one as far as OMe peaks are concerned. The comparative NMR data are given in Table 1. Thus there is general agreement in the spectral data of the synthetic coumarin and exostemin but the m.ps of these two compounds and their acetates differ considerably.



VIII

IX

In exploratory studies of 4-phenylcoumarins with a similar substitution pattern, the simpler analogues of the above coumarins were prepared by condensing appropriate phenols with benzoylacetic ester. In general the properties of these simpler



analogues agreed quite well with the *p*-methoxy derivatives. It is interesting to note that the m.ps of the simpler analogue (X), its acetate and its methyl ether (XI) corresponds closely with those of exostemin, its acetate and methyl ether; this would suggest the possibility of some error of identity of the natural compound. The quinone (XII) has however a much higher m.p. (278–279°) than that of the quinone (II) from exostemin. The m.p. data of these two series of coumarins are given in Table 2 together with those of exostemin and its derivatives.

TABLE 2.

Series	Colour of hydroxy compound	M.pts of			
		Hydroxy compound	Methyl ether	Acetate	5:8-Quinone derivative
Exostemin (Reported data)	Yellow	173–174°	144–145°	203–204°	215–217°
Synthetic Ia	Yellow	195–196°	142–143°	187–188°	214–215°
Synthetic X	Yellow	169–170°	137–138°	206–207°	278–279°

EXPERIMENTAL

Silica gel-G was used throughout for TLC and the developing solvent was benzene:acetone, 80:20.

5-Hydroxy-7-methoxy-4(p-methoxyphenyl) coumarin, (III). A soln of phloroglucinol monomethyl ether (0·7 gm) and *p*-methoxybenzoylacetic ester (1·1 g) in abs alcohol (50 ml) was saturated with dry HCl at 10–15° and kept at room temp for 2 days. The mixture was poured into crushed ice and the solid product (0·45 gm) collected. It was a mixture of two compounds (TLC) and was chromatographed on silica gel (40 gm). Elution with a mixture of benzene–acetone, 96:4 gave the 5-hydroxycoumarin (0·17 gm), colourless needles from MeOH, m.p. 216–218°. (Found: C, 68·5; H, 5·2; $C_{17}H_{14}O_5$ requires: C, 68·5; H, 4·7%); λ_{max}^{MeOH} 260 and 320 mμ; ν_{max}^{Nujol} 3400 cm^{-1} (OH), 1720 cm^{-1} (—O—CO—).

Further elution of the column with benzene–acetone, 90:10, gave the isomeric IV (0·19 g) as colourless needles from MeOH, m.p. 252–254°. (Found: C, 68·3; H, 4·4; $C_{17}H_{14}O_5$ requires: C, 68·5; H, 4·7%); λ_{max}^{MeOH} 260 and 325 mμ; ν_{max}^{Nujol} 3200 cm^{-1} (OH), 1720 cm^{-1} (—O—CO—).

5-Acetoxy-7-methoxy-4-(p-methoxyphenyl) coumarin. Obtained by refluxing a mixture of 5,7-diacetoxy-4-(*p*-methoxyphenyl) coumarin[4] (1 gm), MeI (2 ml) and anhyd K_2CO_3 (3 gm) in dry acetone (150 ml), for 24 hr, this methyl ether acetate (0·8 gm) crystallized from a mixture of EtOAc–pet ether as colourless needles, m.p. 155–157°. (Found: C, 67·5; H, 4·9; $C_{19}H_{16}O_6$ requires: C, 67·1; H, 4·7%), λ_{max}^{MeOH} 315 mμ; ν_{max}^{Nujol} 1720 cm^{-1} (lactone), 1770 cm^{-1} (phenolic ester). On hydrolysis with alcoholic HCl it gave partial methyl ether (III), identical in m.p. and mixed m.p., with the product described earlier.

7-Hydroxy-5,8-dimethoxy-4-(p-methoxyphenyl) coumarin (VII). The Pechmann condensation of 2,6-dibenzyloxy-1,4-dimethoxybenzene (4 gm) and *p*-methoxybenzoylacetic ester (5 gm) was carried out as described earlier by the absolute alcohol–HCl method. The product, isolated after 2 days, crystallized from EtOAc as pale yellow rhombs m.p. 202–204°. (Found: C, 66·2; H, 5·1; $C_{18}H_{16}O_6$ requires: C, 65·9; H, 4·9%), λ_{max}^{MeOH} 265 and 320 mμ; ν_{max}^{Nujol} 3420 cm^{-1} (OH) and 1720 cm^{-1} (lactone). Its acetate prepared by the Ac_2O pyridine method, crystallized from EtOAc as colourless needles, m.p. 135–136°. (Found: C, 64·5; H, 5·1; $C_{20}H_{18}O_7$ requires: C, 64·9; H, 4·9%).

5,7,8-Trimethoxy-4-(p-methoxyphenyl) coumarin (Ib). The above hydroxy coumarin (0·1 gm) was methylated by treatment with etheral diazomethane overnight. The methyl ether (0·1 gm) crystallized from MeOH as colourless needles, m.p. 142–143°. (Found: C, 67·0; H, 5·6; $C_{19}H_{18}O_6$ requires: C, 66·7; H, 5·3%); λ_{max}^{MeOH} 260 and 325 mμ; ν_{max}^{Nujol} 1720 cm^{-1}.

Nitric acid oxidation of the tetramethoxy coumarin (Ib) *to* (p-*benzoquinone*) (II). A soln of the above coumarin (300 mg) in glacial AcOH (3 ml) was treated with HNO_3 (1·2 *d*; 3 ml). The mixture was warmed to 40° when the quinone started separating within 10 min. After keeping at room temp for 1 hr, the quinone was collected and crystallized from EtOAc when it came out as orange red needles (280 mg), m.p. 214–215°. (Found: C, 64·9; H, 4·0; $C_{17}H_{12}O_6$ requires, C, 65·4; H, 3·9%), ν_{max}^{KBr} 1635, 1650, 1700 and 1750 cm^{-1}. It agrees with quinone from exostemin[1] in m.p. and IR.

5,8-*Dihydroxy*-7-*methoxy*-4-(p-*methoxyphenyl*) *coumarin*. The quinone (200 mg) in EtOH (10 ml) was reduced by passing SO_2 gas till the red colour disappeared. The quinol (200 mg), obtained after dilution with water and extraction with chloroform, crystallized from EtOAc as yellow needles, m.p. 205–206°. (Found: C, 64·6; H, 4·0; $C_{17}H_{14}O_6$ requires: C, 65·0; H, 4·4%); λ_{max}^{MeOH} 275 and 315 mμ; ν_{max}^{KBr} 3650 cm^{-1} (OH) and 1720 cm^{-1}. Its diacetate prepared by the Ac_2O–pyridine method at room temp crystallized from EtOAc as colourless plates, m.p. 216–218°. (Found: C, 63·1; H, 4·6; $C_{21}H_{18}O_8$ requires: C, 63·3; H, 4·6%).

1,2-*Dihydroxy*-3,5-*dimethoxybenzene* (IX). A soln of 2-hydroxy-4,6-dimethoxybenzaldehyde (1·8 g) in 1N NaOH aq (10 ml) was treated with aqueous 6% H_2O_2 (10 ml-excess) at 0° in an atmosphere of N_2. The initial ppt formed gradually went into soln and after 1 hr the reaction was complete. Acidification and extraction with chloroform and evaporation of the dried extract gave the catechol as a colourless gum which failed to crystallize. It was, however, a single substance (TLC) and was characterized as its diacetate, prepared by the Ac_2O–pyridine method at room temp. The diacetate crystallized from ether–pet. ether mixture as colourless rhombs, m.p. 101–102°. (Found: C, 56·7; H, 5·7; $C_{12}H_{14}O_6$ requires: C, 56·7; H, 5·5%), λ_{max}^{MeOH} 275 mμ, ν_{max}^{Nujol} 1770 cm^{-1}.

8-*Hydroxy*-5,7-*dimethoxy*-4-(p-*methoxyphenyl*) *coumarin* (Ia). A soln of 1,2-diacetoxy-3,5-dimethoxybenzene (1 gm) and *p*-methoxybenzoylacetic ester (1·3 g) in abs alcohol (50 ml) was saturated with dry HCl at room temp and kept for 2 days. Isolated in the usual manner, the product crystallized from EtOAc as yellow needles, m.p. 195–196°. (Found: C, 66·2; H, 5·0; $C_{18}H_{16}O_6$ requires: C, 65·9; H, 4·9%), λ_{max}^{MeOH} 271 and 319 mμ; ν_{max}^{Nujol} 3450 cm^{-1} (OH), 1730 cm^{-1} (—O—CO—). Sanchez-Viesca *et al.*, reported the m.p. of natural exostemin as 173–174°.

The acetate, prepared by the Ac_2O–pyridine method, crystallized from alcohol as colourless needles, m.p. 187–188°. (Found: 64·9; H, 5·1; $C_{20}H_{18}C_7$ requires: C, 64·9; H, 4·9%), ν_{max}^{Nujol} 1750 and 1770 cm^{-1}. The m.p. reported for exostemin acetate is 203–204° but the IR spectra of these two compounds agree.

The methyl ether, prepared by keeping the 8-hydroxy compound overnight with ethereal diazomethane, crystallized from MeOH as colourless needles which melted alone or when mixed with Ib described earlier at 142–143°.

Oxidation of Ia (5 mg) in AcOH (0·5 ml) with HNO_3 (0·5 ml; 1·2 *d*) at room temp, gave II identical in all respects with the quinone obtained from Ib.

7-*Hydroxy*-5,8-*dimethoxy*-4-*phenylcoumarin* (VI). A well cooled mixture of 2,6-dihydroxy-1,4-dimethoxybenzene (500 mg) and benzoylacetic ester (550 mg), was treated with cold conc H_2SO_4 (1·5 ml). The well mixed liquid was kept in the refrigerator for 24 hr, then poured into crushed ice. The solid product was collected, dried and crystallized from EtOAc when the hydroxycoumarin was obtained as pale yellow plates m.p. 203–205°. (Found: C, 68·1; H, 5·0; $C_{17}H_{14}O_5$ requires: C, 68·5; H, 4·7%); λ_{max}^{MeOH} 265 and 325 mμ; ν_{max}^{KBf} 3300 cm^{-1} (OH) and 1720 cm^{-1} (—O—CO—). Its acetate, obtained by the Ac_2O pyridine method, crystallized from EtOAc–pet. ether as colourless plates, m.p. 119–120°.

5,7,8-*Trimethoxy*-4-*phenylcoumarin* (XI). The methyl ether was obtained by refluxing the above 7-hydroxycoumarin (200 mg) with Me_2SO_4 (0·3 ml) and K_2CO_3 (3 g) in dry acetone soln, for 6 hr. It crystallized from EtOH as pale yellow plates, m.p. 137–138°. (Found: C, 69·5; H, 5·6; $C_{18}H_{16}O_5$ requires: C, 69·2; H, 5·2%), λ_{max}^{MeOH} 260 and 330 mμ; ν_{max}^{KBr} 1720 cm^{-1} (—O—CO—).

Nitric acid oxidation to p-*benzoquinone* (XII). The trimethoxycoumarin (100 mg) when treated with glacial AcOH (2 ml) and HNO_3 (*d* 1·2, 1 ml) at room temp, slowly yelded the quinone in 1 hr. It crystallized from EtOAc as yellow needles, m.p. 278–279°. (Found: C, 68·1; H, 3·9; $C_{16}H_{10}O_5$ requires: C, 68·1; H, 3·5%), λ_{max}^{MeOH} 300 mμ; ν_{max}^{KBr} 1630, 1670 and 1720 cm^{-1}.

8-*Hydroxy*-5,7-*dimethoxy*-4-*phenylcoumarin* (X). To a well cooled mixture of 1,2-diacetoxy-3,5-dimethoxybenzene (1·2 g) and benzoyl acetic ester (0·9 gm) was treated with ice cold conc H_2SO_4 (2 ml) with stirring, kept at 0° for 24 hr and poured into crushed ice. The solid product crystallized from EtOAc as yellow prisms, m.p. 169–170°. (Found: C, 68·2; H, 5·0; $C_{17}H_{14}O_5$ requires: C, 68·5; H, 4·7%), λ_{max}^{MeOH} 270 and 325 mμ; ν_{max}^{Nujol} 3550 cm^{-1} (OH), 1720 cm^{-1} (lactone). Its acetate prepared by the Ac_2O–pyridine method, came out of EtOAc as colourless needles, m.p. 206–207°. (Found: C, 66·8; H, 4·8; $C_{19}H_{16}O_6$ requires: C, 67·1; H, 4·7%), ν_{max}^{Nujol} 1750 and 1770 cm^{-1}.

On methylation with diazomethane, the 8-hydroxycoumarin, gave the trimethyl ether, m.p. and mixed m.p. with XI described earlier, 137–138°.

On oxidation with HNO_3 in glacial AcOH, X also gave XI identical with the quinone described earlier.

REFERENCES

[1] F. Sanchez-Viesca, E. Diaz and G. Chavez, *Sobretiro de Ciencia, Mex.*, XXV(4), 135 (1967).
[2] A. Robertson and T. S. Subramanian, *J. Chem. Soc.* 288 (1937).
[3] R. L. Shriner and A. G. Schmidt, *J. Am. Chem. Soc.* **51**, 3636 (1929).
[4] Sonn, *Ber. Dtsch. Chem. Ges.* **51**, 825 (1918).
[5] V. K. Ahluwalia, G. P. Sachdev and T. R. Seshadri, *Indian J. Chem.* in press.
[6] J. R. Clarke and A. Robertson, *J. Chem. Soc.* 302 (1949).
[7] H. Appel, *Ibid.* 1031 (1935).
[8] Ishwar Dass, N. Narsimhachari and T. R. Seshadri, *Proc. Ind. Acad. Sci.* **37A**, 599 (1953).