[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE STATE UNIVERSITY]

Naturally Occurring Oxygen Heterocyclics. III.[1] The Structure of the Isoflavone Tlatlancuayin[2]

BY PIERRE CRABBÉ,[3a] P. R. LEEMING[3b] AND CARL DJERASSI

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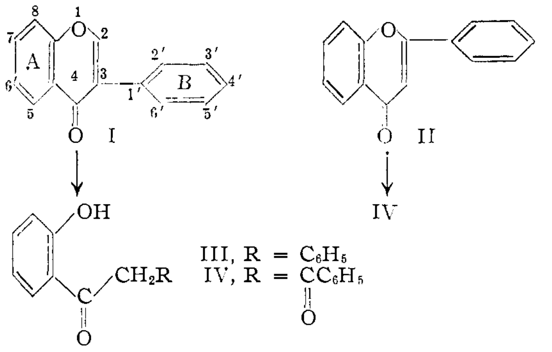
Degradation experiments are recorded which demonstrate that tlatlancuayin is 2',5-dimethoxy-6,7-methylenedioxyisoflavone (XII), the first naturally occurring isoflavone with a sole 2'-methoxy substituent in ring B. This constitutes also the first instance in the isoflavone series where a methylenedioxy grouping has been observed in ring A.

Extensive work[4] in this Laboratory on the sesquiterpene iresin of *Iresine celosioides* L. (fam. *Amarantaceae*),[5] a shrub known in central Mexico as "tlatlancuaya," necessitated the processing of large amounts of this plant. In the course of the isolation work, there was encountered in about 0.005% yield a beautifully crystalline substance, whose empirical formula and spectral properties demonstrated clearly that it had no structural connection with the sesquiterpene iresin,[4,5] which represents the main constituent of this extract. We have termed the new substance "tlatlancuayin" after the indigenous name[6] of the plant and the present communication is concerned with its structure elucidation.

Tlatlancuayin forms colorless crystals (m.p. 147–148°) and its empirical formula corresponds to $C_{18}H_{14}O_6$, indicating that the substance must be substantially aromatic. The spectral data suggested membership in the group of flavonoids and functional group analysis pointed toward the presence of two methoxyl functions and one methylene dioxy grouping. Since the latter is comparatively rare among flavonoids, it appeared of interest to establish precisely the substitution pattern of tlatlancuayin and as will become apparent from the sequel, this proved to be unique among isoflavones.

The infrared spectrum[7] exhibited strong, multiple absorption in the 6–7 μ region (for details see Experimental) compatible with a flavone or isoflavone structure but its ultraviolet absorption spectrum (Fig. 1) was more suggestive of an isoflavone (I)[8] rather than a flavone (II),[9,10] although it was appreciated[8] that variations in the substitution pattern could produce marked spectral changes.



III, R = C_6H_5
IV, R = $\underset{\overset{\|}{O}}{C}C_6H_5$

The simplest means of differentiation between an isoflavone (I) and a flavone (II) is the examination of the products of relatively mild alkaline treatment,[8,10,11] since the former (I) furnishes a benzyl *o*-hydroxyphenyl ketone (III) with loss of carbon (as formic acid), while a flavone (II) leads to an *o*-hydroxydibenzoylmethane (IV) which can then undergo further β-diketone cleavage.

When tlatlancuayin was heated under reflux with 5% aqueous sodium hydroxide solution,[12] there was isolated in excellent yield a yellow crystalline ketone, m.p. 115–116°. While formic acid *per se* was not isolated, the empirical formula ($C_{17}H_{16}O_6$) strongly indicated that it was a substituted benzyl *o*-hydroxyphenyl ketone (III). Thus, its ultraviolet absorption spectrum (Fig. 1) exhibited three well characterized maxima, typical of the substituted desoxybenzoin moiety, and these were also found in dihydrotlatlancuayin (subsequently shown to be XXIIa) in which the 2,3-double bond (see I) had been reduced. The infrared spectrum of this cleavage product, discussed in the Experimental section in connection with certain model substances, further supported this deduction. Unambiguous proof for the desoxybenzoin structure of the alkaline cleavage product—and hence of the isoflavone nature of tlatlancuayin—was provided by condensing the cleavage product with ethyl formate,[13]

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[1] Paper II, C. Djerassi, E. J. Eisenbraun, B. Gilbert, A. J. Lemin, S. P. Marfey and M. P. Morris, THIS JOURNAL, **80**, 3686 (1958).

[2] Supported by research grant No. H-2574 from the National Heart Institute of the National Institutes of Health, U. S. Public Health Service.

[3] (a) Recipient of a Fulbright Travel Grant from the U. S. Educational Foundation in Belgium and Postdoctorate research fellow 1957–1958; (b) Postdoctorate research fellow 1956–1957; present address: Dyson Perrins Laboratory, Oxford University.

[4] For latest paper see C. Djerassi and S. Burstein, THIS JOURNAL, **80**, 2593 (1958), and P. Crabbé, S. Burstein and C. Djerassi, *Bull. Soc. Chim. Belg.*, **67**, in press (1958).

[5] C. Djerassi, P. Sengupta, J. Herran and F. Walls, THIS JOURNAL, **76**, 2966 (1954).

[6] As pointed out by M. Martinez ("Catalogo de Nombers Vulgares y Cientificos de Plantas Mexicanas," Ediciones Botas, Mexico, D. F., 1937) the name "tlatlancuaya" refers to various *Iresine* species and it has already been mentioned in the famous 1552 manuscript "Libellus de Medicinalibus Indorum Herbis" (see 1952 edited version by Francisco Guerra, Editorial Vargas Rea y El Diario Español, Mexico, D. F.).

[7] The infrared spectra of some flavones have been recorded by H. L. Hergert and E. F. Kurth, THIS JOURNAL, **75**, 1622 (1953).

[8] See W. K. Warburton, *Quart. Rev.*, **8**, 67 (1954).

[9] S. Aronoff, *J. Org. Chem.*, **5**, 561 (1940).

[10] See C. Sannié and H. Sauvain "Les Couleurs des Fleurs et des Fruits. Anthocyannes et Flavones," Mém. Muséum National d'Histoire Naturelle, Série B, Botanique, Vol. II, Editions du Muséum, Paris, 1952.

[11] For a brief review see also K. P. Link in H. Gilman's "Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1943, 2nd edit., Vol. II, pp. 1331–1339.

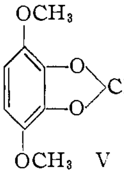
[12] These conditions were employed by A. Robertson, C. W. Suckling and W. B. Whalley (*J. Chem. Soc.*, 1571 (1949)) in the cleavage of trimethylsantal (5,7,3',4'-tetramethoxyisoflavone).

[13] This synthesis of isoflavones from benzyl *o*-hydroxyphenyl ketones is due to E. Späth and E. Lederer (*Ber.*, **63**, 743 (1930)) and has been improved by H. S. Mahal, H. S. Rai and K. Venkataraman (*J. Chem. Soc.*, 1120, 1769 (1934)).

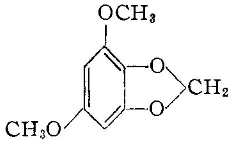
whereupon tlatlancuayin was re-formed in good yield, presumably[14] *via* the intermediate 2-hydroxy-2,3-dihydrotlatlancuayin (XXIIb).[15]

With the establishment of the isoflavanoid nature of tlatlancuayin, there remained only the location of the two methoxyl groups and the methylenedioxy function for the complete structure elucidation of this substance. Since alkali fusion usually results in rupture of the molecule[8,11] with formation of a substituted phenylacetic acid (derived from ring B of I), tlatlancuayin was subjected to such treatment and yielded salicylic acid together with *o*-methoxyphenylacetic acid (XIX). The former is a typical degradation product (arising from an unsubstituted ring A) of a flavone (II)[10,11] rather than an isoflavone (I), but it was soon noted that alkali fusion of synthetic[16] *o*-methoxyphenylacetic acid (XIX) also gave some salicylic acid. Consequently, both acids are derived from ring B of tlatlancuayin and the latter can now be defined as a 2′-methoxyisoflavone with the attachment of the second methoxy and the methylenedioxy substituents limited to positions 5, 6, 7 and 8.

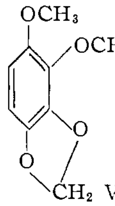
More stringent alkali treatment[17] of a substituted benzyl *o*-hydroxyphenyl ketone (III) leads to cleavage and formation of a substituted phenylacetic acid (*vide supra*) and a phenol, the latter representing ring A of the isoflavone I. The desoxybenzoin (later shown to be XXIIIa) derived from tlatlancuayin responded to these conditions[17] in the expected manner to provide *o*-methoxyphenylacetic acid (XIX) and x-methoxy-y,z-methylenedioxyphenol, the presence of the intact methylenedioxy grouping having been confirmed by the positive Hansen[18] and Lebat[19] tests. Methylation of this phenol (m.p. 88–89°) yielded the corresponding methyl ether (m.p. 40–40.5°) which could have only one of the four possible structures, V, VI, VII or VIII.



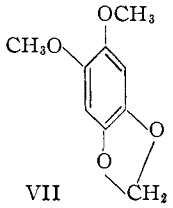
V



VIII



VI



VII

The phenol and its methyl ether exhibited an infrared absorption band in the 12.4 μ region, suggestive of a 1,2,3,4-tetrasubstituted benzene derivative,[20] but as will be shown below such infrared deductions are apparently not applicable to this series. The two 1,2,3,4-tetrasubstituted isomers V (m.p. 79°; dibromide, m.p. 100°) and VI (oily; dibromide, m.p. 92°) already have been reported in the literature[21] and their physical constants differ sufficiently from those of our methyl ether (m.p. 40°; dibromide, oily) to preclude identity. Consequently, there remain four structural alternatives—IX (yielding VII), X, XI or XII (the latter three furnishing the methyl ether VIII)—for tlatlancuayin. A differentiation among these four possibilities cannot be made on the basis of biogenetic probability since the substitution pattern of tlatlancuayin is practically unique. Thus, it represents the first isoflavone with a 2′-methoxy substituted ring B[22] and among all flavonoids,[23] only one is known[24] which possesses a methylenedioxy function in ring A. Therefore, while 5,6,7-trioxygenated isoflavones (analogous to X and XII) are known[25] in contrast to 5,6,8-substituted ones (corresponding to IX and XI), the latter pattern has been observed frequently in the flavone series[23] and it was obvious, therefore, that chemical proof had to be provided to settle this outstanding point.

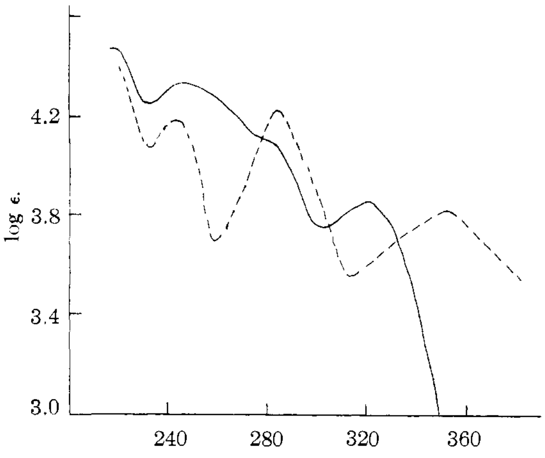


Fig. 1.—Ultraviolet spectra in 95% ethanol: tlatlancuayin (XII), ———; *o*-methoxybenzyl 2-methoxy-3,4-methylenedioxy-6-hydroxyphenyl ketone (XXIIIa), --------.

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[14] For pertinent discussion see ref. 8 and (a) W. B. Whalley, THIS JOURNAL **75**, 1059 (1953); (b) M. L. Wolfrom, J. E. Mahan, P. W. Morgan and G. F. Johnson, *ibid.*, **63**, 1248 (1941).

[15] The intermediate was not fully characterized, but it melted considerably higher than tlatlancuayin, gave no color with ferric chloride and upon heating with chloroform–methanol was transformed into tlatlancuayin.

[16] J. Levine, T. E. Eble and H. Fischbach, THIS JOURNAL, **70**, 1930 (1948).

[17] *Cf.* W. Baker, *J. Chem. Soc.*, 1022 (1928).

[18] O. R. Hansen, *Acta Chem. Scand.*, **7**, 1125 (1953).

[19] M. A. Lebat, *Bull. soc. chim. France*, 745 (1909).

[20] See D. H. Whiffen and H. W. Thompson, *J. Chem. Soc.*, 268 (1945); P. L. Launer and D. A. McCaulay, *Anal. Chem.*, **23**, 1875 (1951); R. L. Werner, W. Kennard and D. Rayson, *Austral. J. Chem.*, **8**, 346 (1955).

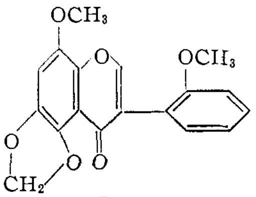
[21] G. Ciamician and P. Silber, *Ber.*, **29**, 1799 (1896); W. Baker and H. A. Smith, *J. Chem. Soc.*, 2542 (1931); W. Baker and R. I. Savage, *ibid.*, 1602 (1938).

[22] Methylisogenistein and isogenistein (from soya beans) were believed to be 2′-hydroxyisoflavones (K. Okano and I. Beppu, *J. Agr. Chem. Soc. Japan*, **15**, 645 (1939)), but this has been disproved by W. B. Whalley (ref. 14a) and by W. Baker, J. B. Harborne and W. D. Ollis (ref. 27).

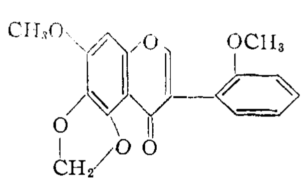
[23] A very complete list of naturally occurring flavonoids including botanical sources is given by T. A. Geissman and E. Hinreiner, *Botan. Rev.*, **18**, 77 (1952).

[24] Meliternatin has been shown to be 3,5-dimethoxy-6,7,3′,4′-bismethylenedioxyflavone (L. H. Briggs and R. H. Locker, *J. Chem. Soc.*, 3131 (1951)).

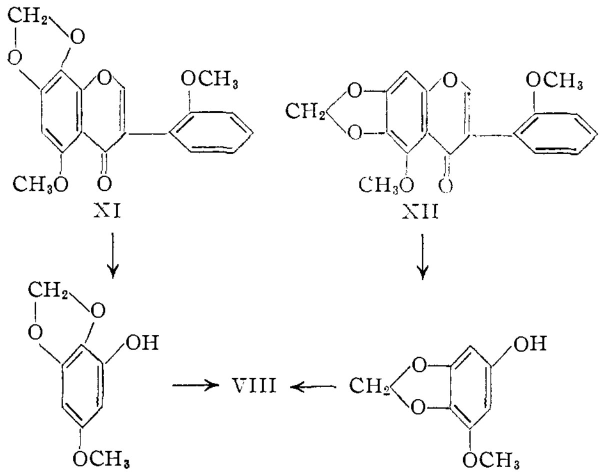
[25] (a) Muningin: F. E. King, T. J. King and A. J. Warwick, *ibid.*, 96 (1952); (b) irigenin: W. Baker and R. Robinson, *ibid.*, 152 (1929); (c) tectorigenin: R. L. Shriner and R. W. Stephenson, THIS JOURNAL **64**, 2737 (1942).



IX



X



It has been reported[24,26] that methoxyl groups at C-5 can be demethylated with aluminum chloride in boiling ether and this reaction yielded indeed a monomethoxy derivative of tlatlancuayin. The latter, on remethylation, regenerated tlatlancuayin thus demonstrating that the methylenedioxy function had not been affected by this treatment. In order to preclude the possibility that demethylation may have occurred at position 2′ in ring B, the partially demethylated derivative (subsequently shown to be XXIV) was boiled with 16% aqueous alkali, whereupon *o*-methoxyphenylacetic acid (XIX) was isolated. This sequence proves that demethylation must have occurred in ring A and on the basis of earlier work[24,26] the methoxyl group can be placed at C-5 eliminating structures IX and X for tlatlancuayin.

The simplest way of distinguishing between the remaining two structural alternatives (XI and XII) would be the identification of the cleavage phenol as either XIII (derived from XI) or XIV (derived from XII). Neither one of these phenols is known and since an unambiguous synthesis would have involved a fairly long sequence, the following indirect approach was employed. Total demethylation of tlatlancuayin and cleavage of the methylenedioxy grouping was accomplished by means of aluminum chloride in refluxing benzene solution[27] and led to the corresponding tetrahydroxy isoflavone which had to possess structure XVa or XVIa.[28] The latter was completely methylated with dimethyl sulfate and potassium carbonate in acetone solution to the corresponding tetramethoxyisoflavone which had to be either XVb or XVIb. While neither product has been described in the literature, alkali cleavage *via* the desoxybenzoin XVII or XVIII now offers a means of differentiation since in addition to the common cleavage product *o*-methoxyphenylacetic acid (XIX), the 2′,5,7,8-tetramethoxyisoflavone (XVb) should yield 2,3,5-trimethoxyphenol (XX), while the 2′,5,6,7-tetramethoxy isomer XVIb should give 3,4,5-trimethoxyphenol ("antiarol")(XXI).[29] In actual fact, rupture of the molecule with alkali furnished *o*-methoxyphenylacetic acid (XIX) and antiarol (XXI), from which it follows that the immediate precursors are the desoxybenzoin XVIII, the 2′,5,6,7-tetrasubstituted isoflavones XVIa and XVIb, and that tlatlancuayin should therefore be represented as 2′,5-dimethoxy-6,7-methylenedioxyisoflavone (XII). The product of the mild alkaline cleavage must, *ipso facto*, be *o*-methoxybenzyl 2-methoxy-3,4-methylenedioxy-6-hydroxyphenyl ketone (XXIIIa); the further alkaline degradation product is 3-methoxy-4,5-methylenedioxyphenol (XIV) and its methyl ether, 1,3-dimethoxy-5,6-methylenedioxybenzene (VIII). Some derivatives of the desoxybenzoin XXIIIa and in particular comments on the spectral shifts observed upon methylation (XXIIIb) and acetylation (XXIIIc) are recorded in the Experimental section.

Tlatlancuayin (XII) is the first flavonoid isolated from a member of the *Amarantaceae* plant family. It is the only known natural 2′-methoxyisoflavone[22] and even among the much more abundant flavones and flavanones there had been described only one representative each[30] bearing a single methoxyl or hydroxyl group at position 2′ in ring B. One other isoflavone—ψ-baptigenin[31]—has been reported which possesses a methylenedioxy grouping, but this is present in ring B as has already been observed in the flavone series.[32] On the other hand, as far as such substitution in ring A is concerned, meliternatin[24] and tlatlancuayin (XII) are the only flavonoids for which such a structural feature has been established. The biogenesis of the carbon skeleton of the flavonoids has already been discussed in detail[23,33] and is of the C_6–C_3–C_6

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[26] W. Baker and W. H. C. Simmonds, *J. Chem. Soc.*, 1370 (1940), and earlier papers.

[27] *Cf.* W. Baker, J. B. Harborne and W. D. Ollis, *ibid.*, 1860 (1953); R. N. Iyer, K. H. Shah and K. Venkataraman, *Proc. Indian Acad. Sci.*, **33**, 228 (1951).

[28] Certain 5-methoxylated isoflavones are known to rearrange in the alternate direction upon demethylation, especially with hydriodic acid (W. B. Whalley, *J. Chem. Soc.*, 3366 (1953); W. Baker, L. Dunstan, J. B. Harborne, W. D. Ollis and R. Winter, *Chemistry & Industry*, 277 (1953); D. M. Donnelly, E. M. Philbin and T. S. Wheeler, *ibid.*, 567 (1953)) similar to the corresponding rearrangement in the flavone series (F. Wessely and G. H. Moser, *Monatsh.*, **56**, 97 (1930)). If this obtained also in the demethylation of tlatlancuayin, then the 2′,5,6,7-tetrahydroxyisoflavone (XVIa) could have arisen from structure XI, but this is not very likely since no rearrangement was observed in the demethylation of tlatlancuayin with aluminum chloride in ether (as proved by remethylation to tlatlancuayin). Furthermore, such rearrangements with aluminum chloride seem to have been observed in the isoflavone series only with C-methylated derivatives and it has been suggested (see T. S. Wheeler, *Rec. Chem. Progress*, **18**, 133 (1957)) that this involves direct migration of such a C-methyl group.

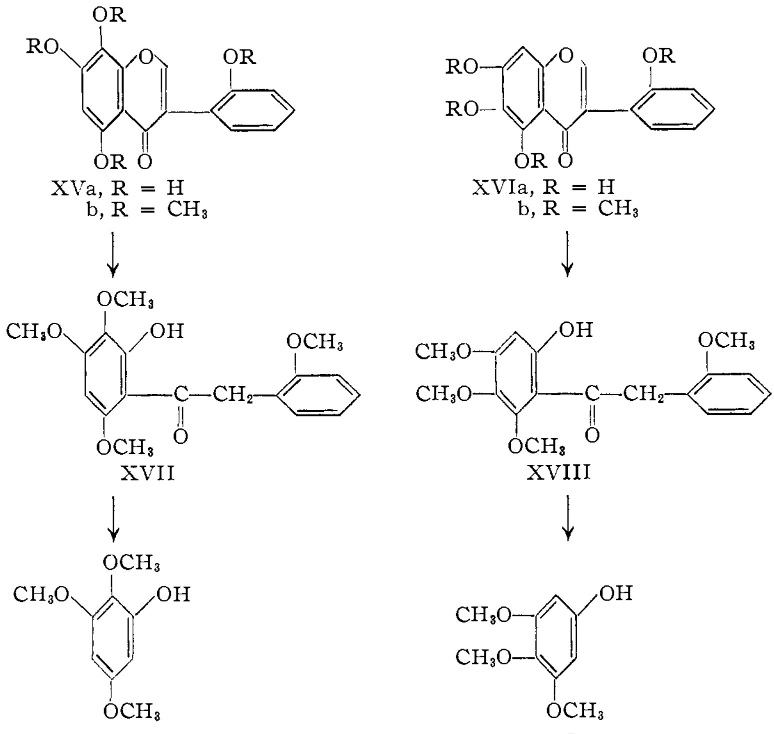
[29] E. Chapman, A. G. Perkin and R. Robinson, *J. Chem. Soc.*, 3028 (1927).

[30] (a) Citronetin (2′-methoxy-5,7-dihydroxyflavanone): T. H. Simpson and W. B. Whalley, *ibid.*, 166 (1955)) and references cited; (b) datiscetin (2′,3,5,7-tetrahydroxyflavone): J. Kalff and R. Robinson, *ibid.*, 1968 (1925).

[31] W. Baker, R. Robinson and N. M. Simpson, *ibid.*, 805 (1937), and earlier references.

[32] *E.g.* Meliternin (3′,4′-methylenedioxy-3,5,7,8-tetramethoxyflavone): L. H. Briggs and R. H. Locker, *ibid.*, 2157 (1949).

[33] R. Robinson "The Structural Relations of Natural Products," Clarendon Press, Oxford, 1955, pp. 36 *et seq.*



XVa, R = H
b, R = CH_3

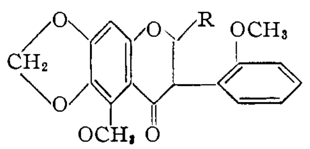
XVIa, R = H
b, R = CH_3

XVII

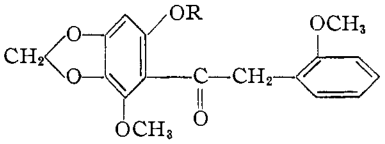
XVIII

XX

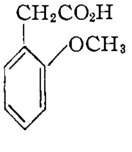
XXI



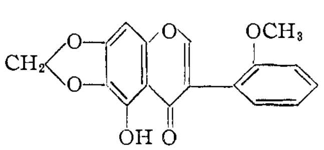
XXIIa, R = H
b, R = OH



XXIIIa, R = H
b, R = CH_3
c, R = CH_3CO



XIX



XXIV

type. In that connection, Birch[34] has analyzed the oxygenation pattern among flavonoids and has noted the comparatively rare occurrence of oxygen at positions 2′ and 6 as compared to the high incidence of oxygenation at positions 4′, 5 and 7. The introduction or removal of oxygen is a very likely biological process and has been invoked[34] to explain the unexpected presence or absence of oxygen. With that viewpoint in mind, the oxygenation pattern observed in tlatlancuayin (XII) represents an example of introduction (C-6) as well as removal (C-4′) of oxygen.

Certain isoflavones, notably genistein (4′,5,7-trihydroxyisoflavone) are reported[35] to exhibit estrogenic activity. Consequently, tlatlancuayin (XII) and its 5-demethyl derivative (XXIV) were submitted to estrogenic assay[36] in mice. By injection—using uterine weight as the criterion and estrone as the standard–tlatlancuayin exhibited approximately one-ten thousandth the activity of estrone while XXIV was somewhat less active.

Acknowledgment.—We wish to express our sincere appreciation to our colleague Dr. B. Gilbert for many helpful suggestions and to several other investigators for providing us with certain model substances for spectral comparisons: W. Baker (University of Bristol), D. J. Cram (U. C. L. A.), F. E. King and J. W. W. Morgan (British Celanese Ltd.), W. D. Ollis (University of Bristol) and W. B. Whalley (University of Liverpool).

Experimental[37]

Isolation of Tlatlancuayin (XII).—The total plant (*Iresine celosioides*) was processed as described earlier[5] including precipitation with lead acetate and in this manner there was obtained 540 g. of viscous oil from 100 kg. of fresh plant. Thorough extraction with two 4-l. portions of chloroform removed 394 g. of oil. This was stirred with 2 l. of 3:1 benzene–chloroform, whereupon 225 g. of oil dissolved and this was chromatographed on 8 kg. of alumina deactivated with 400 cc. of 10% aqueous acetic acid. Elution with the same solvent removed 17 g. of very crude tlatlancuayin, while further development of the column with chloroform containing 2% methanol yielded iresin.[5] Rechromatography of the tlatlancuayin fraction and recrystallization from methanol afforded colorless, lustrous hexagons, m.p. 147–148° (5 g.), ultraviolet absorption spectrum (Fig. 1) in ethanol with maxima at 245 mμ (log ε 4.34) and 320 mμ (log ε 3.86) and inflection at 278 mμ (log ε 4.12), $\lambda_{max}^{CHCl_3}$ 6.02, 6.08, 6.15, 6.20 and 6.26 μ,[38] negative ferric chloride test, blue-violet Hansen[18] and greenish-blue Lebat[19] tests for methylenedioxy group.

Anal. Calcd. for $C_{18}H_{14}O_6$: C, 66.25; H, 4.32; O, 29.42; 2-CH_3O, 19.00; mol. wt., 326. Found: C, 66.21; H, 4.52; O, 29.41; CH_3O, 18.88;[39] C–CH_3, 0.0; Rast mol. wt., 322.

Dihydrotlatlancuayin (XXIIa).—Hydrogen up-take corresponding to one equivalent stopped after 42 min. when 163 mg. of tlatlancuayin was shaken in 100 cc. of ethanol with 30 mg. of 5% palladium–charcoal catalyst in an atmosphere of hydrogen. The catalyst was filtered, the filtrate evaporated to dryness and the residue passed over 3 g. of alumina. Elution with benzene and recrystallization from methylene chloride–hexane provided 140 mg. of dihydrotlatlancuayin,

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[34] A. J. Birch in L. Zechmeister's "Progress in the Chemistry of Organic Natural Products," Springer, Vienna, 1957, Vol. XIV, pp. 186–216.

[35] R. B. Bradbury and D. E. White, *J. Chem. Soc.*, 871 (1953).

[36] Dr. R. I. Dorfman, Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

[37] Melting points were determined on the Kofler block. We are indebted to Dr. H. Kovacs for the infrared spectra, to Mr. K. Hutchinson for the ultraviolet absorption spectra and to Dr. A. Bernhardt, Mülheim, Germany, for the microanalyses.

[38] The infrared spectrum of 3′,4′-methylenedioxy-5,7-dihydroxyisoflavone was determined for comparison: λ_{max}^{Nujol} 6.02, 6.16 and 6.34 μ (W. Baker, J. B. Harborne and W. D. Ollis, *J. Chem. Soc.*, 1856 (1953)).

[39] A methylenedioxy group does not interfere as shown in a blank experiment with piperonal.

m.p. 89–91°; $\lambda_{\max}^{\text{EtOH}}$ 242, 280 and 338 mμ (log ϵ 4.27, 4.13, 3.71); $\lambda_{\max}^{\text{CHCl}_3}$ 5.92, 6.08, 6.16 and 6.23 μ.

Anal. Calcd. for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91; O, 29.24; 2 CH_3O, 18.89. Found: C, 66.03; H, 4.89; O, 29.26; CH_3O, 18.76.

Dihydrotlatlancuayin oxime was produced in ethanol solution (3 hr., steam-bath) with hydroxylamine hydrochloride and sodium acetate. Recrystallization from methylene chloride–methanol yielded small needles with m.p. 257–259°.

Anal. Calcd. for $C_{18}H_{17}NO_6$: C, 62.97; H, 4.99; N, 4.08; O, 27.96. Found: C, 62.36; H, 5.11; N, 4.65; O, 27.59.

Alkali Fusion of Tlatlancuayin (XII).—Sodium hydroxide (2.0 g.) and 0.6 cc. of water were heated in a nickel crucible to 120°, 0.5 g. of tlatlancuayin was added slowly with stirring and the yellow mixture was heated to 220° where it was kept for 15 min. The mixture turned to a greenish-black tar at 160° while at 200° it became a greenish-yellow sand. After cooling, water was added, the solution was extracted with ether and separated into neutral (9 mg.), phenolic (25 mg.) and acidic (164 mg.) oily fractions. The acidic material was submitted to slow sublimation at 3 mm. and 100–120° whereupon 30 mg. of long colorless needles was obtained which on further recrystallization exhibited m.p. 154–156°, undepressed upon admixture with **salicylic acid**. Identity was confirmed by infrared spectral comparison.

Anal. Calcd. for $C_7H_6O_3$: C, 60.87; H, 4.38; O, 34.75; neut. equiv., 138. Found: C, 60.79; H, 4.62; O, 34.75; neut. equiv., 138.

The residue of the above sublimation was kept at 120–130° and 0.1 mm. whereupon a few mg. of **o-methoxyphenylacetic acid (XIX)** was obtained. When the fusion temperature was kept below 200°, this acid proved to be the major constituent of the acidic fraction and after crystallization from benzene–hexane melted at 122–123°. Identification with an authentic sample[16] was accomplished by mixture melting point determination and infrared comparison.

Anal. Calcd. for $C_9H_{10}O_3$: C, 65.05; H, 6.07; neut. equiv., 166. Found: C, 65.09; H, 6.27; neut. equiv., 159.

Alkali fusion of 0.5 g. of synthetic *o*-methoxyphenylacetic acid (XIX)[16] under the above conditions furnished 67 mg. of salicylic acid, m.p. 153–156°.

***o*-Methoxybenzyl 2-Methoxy-3,4-methylenedioxy-6-hydroxyphenyl Ketone (XXIIIa).**—A mixture of 1.0 g. of tlatlancuayin, 2.5 g. of sodium hydroxide and 50 cc. of water was heated under reflux for 1.5 hr. at which time a yellow, homogeneous solution was present. After cooling and acidification with dilute sulfuric acid, a yellow precipitate formed which was collected and recrystallized from methylene chloride–hexane to yield 0.97 g. of yellow crystals, m.p. 113–115°. Further recrystallization afforded the analytical sample, m.p. 115–116°, ultraviolet absorption spectrum (Fig. 1)[40] in ethanol with maxima at 243, 284 and 352 mμ, log ϵ 4.20, 4.24 and 3.83. The infrared spectrum ($CHCl_3$) exhibited a broad band at 6.09 μ with a shoulder at 6.17 μ, very similar to that of synthetic[14a] *o*-methoxybenzyl 2,4-dimethoxy-6-hydroxyphenyl ketone. The cleavage product gave a green color with ferric chloride, a purple one in the Hansen test[18] and a greenish-blue color in the Lebat reaction.[19]

Anal. Calcd. for $C_{17}H_{16}O_6$: C, 64.55; H, 5.10; O, 30.35; 2 CH_3O, 19.61. Found: C, 64.48; H, 5.44; O, 30.32; CH_3O, 19.10.

The oxime crystallized as fine needles, m.p. 138–139°, from ether–hexane.

Anal. Calcd. for $C_{17}H_{17}NO_6$: C, 61.63; H, 5.17; N, 4.23; O, 28.98. Found: C, 61.65; H, 5.34; N, 4.31; O, 28.71.

The methyl ether XXIIIb was obtained in nearly quantitative yield when 145 mg. of the desoxybenzoin XXIIIa was heated under reflux for 37 hr. with 0.2 cc. of freshly distilled dimethyl sulfate, 700 mg. of anhydrous potassium carbonate and 10 cc. of acetone (distilled over potassium carbonate). The neutral product, obtained upon ether extraction, was purified by chromatography on 15 g. of Merck acid-washed alumina, elution with benzene and recrystallization from ether–hexane. The methyl ether formed dimorphic crystals, silky plates (m.p. 54–56°) and long needles (m.p. 79–80°), the transformation of which can be observed readily under a microscope. The infrared spectrum ($CHCl_3$ solution) showed bands at 5.84 μ (non-hydrogen bonded phenyl ketone)[41] and 6.10 μ, while its ultraviolet spectrum (ethanol solution, unchanged in alkali) exhibited[42] maxima at 274 mμ (log ϵ 3.77) and 279 mμ (log ϵ 3.77) with an inflection at 296 mμ (log ϵ 3.62).

Anal. Calcd. for $C_{18}H_{18}O_6$: C, 65.44; H, 5.49; O, 29.06; 3 CH_3O, 28.01. Found: C, 65.28; H, 5.52; O, 28.93; CH_3O, 27.89.

The acetate XXIIIc was prepared in acetic anhydride–pyridine at room temperature and recrystallized from ether–hexane, whereupon it exhibited m.p. 67–69°, $\lambda_{\max}^{\text{EtOH}}$ 273 mμ (log ϵ 3.83), $\lambda_{\text{inflect.}}^{\text{EtOH}}$ 278 mμ (log ϵ 3.81); $\lambda_{\max}^{\text{CHCl}_3}$ 5.62, 5.86 and 6.09 μ.

Anal. Calcd. for $C_{19}H_{18}O_7$: C, 63.68; H, 5.06; O, 31.25. Found: C, 63.82; H, 5.04; O, 30.95.

When the acetylation was carried out[25a] with fused sodium acetate in boiling acetic anhydride, the **enol acetate** of the acetate XXIIIc was produced. After chromatographic purification and recrystallization from methylene chloride–hexane, it melted at 105–108°; m.p. 159–161° after drying in a high vacuum, $\lambda_{\max}^{\text{CHCl}_3}$ 5.63 μ (very strong) and 6.09 μ, but no band in the 5.8–5.9 μ region.

Anal. Calcd. for $C_{21}H_{20}O_8$: C, 62.99; H, 5.04; O, 31.97. Found: C, 63.14; H, 4.94; O, 31.64.

Synthesis of Tlatlancuayin (XII) from *o*-Methoxybenzyl 2-Methoxy-3,4-methylenedioxy-6-hydroxyphenyl Ketone (XXIIIa).—A solution of 600 mg. of the ketone XXIIIa in 30 cc. of redistilled, dry ethyl formate was added slowly to 1.0 g. of powdered sodium at −10°. After 24 hr. below 0° and 48 hr. at room temperature ice-water was added and the solution was extracted with ether and chloroform to yield 588 mg. of solid (m.p. 168–173°, negative reaction with ferric chloride), raised to 170–174° upon recrystallization from methanol–methylene chloride. This substance is probably[14] the 2-hydroxyisoflavanone XXIIb and upon further recrystallization from methanol–chloroform (traces of hydrogen chloride present?) lowered the melting point to 145–147° (442 mg.), undepressed upon admixture with tlatlancuayin (XII). The infrared spectrum proved to be completely identical with that of the natural product.

Anal. Calcd. for $C_{18}H_{14}O_6$: C, 66.25; H, 4.32; O, 29.43. Found: C, 66.22; H, 4.41; O, 29.28.

Infrared Spectral Evidence for Methylenedioxy Grouping.[43]—The following intense bands (chloroform solution) appear to be associated with this substituent: tlatlancuayin (XII): 8.60, 8.91, 9.06, 9.28, 9.51 μ; dihydrotlatlancuayin (XXIIa): 8.57, 8.82, 9.02, 9.38, 9.44 μ; desoxybenzoin (XXIIIa): 8.56, 8.90, 8.97, 9.15, 9.50 μ; desoxybenzoin methyl ether (XXIIIb): 8.74, 8.94, 9.17, 9.46 μ; desoxybenzoin acetate (XXIIIc): 9.04, 9.22, 9.49 μ; enol acetate of desoxybenzoin acetate (XXIIIc): 8.92, 9.08, 9.17, 9.43 μ; 5,7-dihydroxy-3′,4′-methylenedioxyisoflavone[38] (in Nujol): 8.60, 9.03, 9.44, 9.66 μ; 3,4-methylenedioxybenzyl 2,4,6-trihydroxyphenyl ketone[40] (in Nujol): 8.53, 9.02, 9.28, 9.60 μ; piperonal: 9.06, 9.15, 9.60 μ.

Alkali Cleavage of *o*-Methoxybenzyl 2-Methoxy-3,4-methylenedioxy-6-hydroxyphenyl Ketone (XXIIIa).—A mix-

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[40] The ultraviolet spectra of the following model compounds were measured: *o*-methoxybenzyl 2,4-dimethoxy-6-hydroxylphenyl ketone (W. B. Whalley ref. 14), $\lambda_{\max}^{\text{EtOH}}$ 285 mμ (log ϵ 4.56), $\lambda_{\text{inflect.}}^{\text{EtOH}}$ 324 mμ (log ϵ 3.84); *o*-methoxybenzyl 2,4,6-trihydroxyphenyl ketone (W. B. Whalley, ref. 14), $\lambda_{\max}^{\text{EtOH}}$ 285 mμ (log ϵ 4.45), $\lambda_{\text{inflect.}}^{\text{EtOH}}$ 325 mμ (log ϵ 3.70); 3,4-methylenedioxybenzyl 2,4,6-trihydroxyphenyl ketone (W. Baker, J. B. Harborne and W. D. Ollis, ref. 38), $\lambda_{\max}^{\text{EtOH}}$ 291 mμ (log ϵ 4.53), $\lambda_{\text{inflect.}}^{\text{EtOH}}$ 318 mμ (log ϵ 4.14).

[41] This band is also observed with synthetic *o*-methoxybenzyl 2,4,6-trimethoxyphenyl ketone (ref. 14a) and 2,4-dimethoxybenzyl 2,4,6-trimethoxyphenyl ketone (F. E. King and K. G. Neill, *J. Chem. Soc.*, 4752 (1952)) while the position of the second band (doublet) is shifted closer to 6.2 μ since it is very dependent on the type of methoxy substitution.

[42] The above two models (ref. 41) also show a maximum in the 275 mμ region which, however, is not so well resolved and there is no shoulder near 300 mμ.

[43] H. Tschamler and R. Leutner, *Monatsh.*, **83**, 1502 (1952); H. Tschamler, *Spectr. Acta*, **6**, 95 (1952).

ture of 400 mg. of the ketone XXIIIa, 5.0 g. of potassium hydroxide and 40 cc. of water was heated under reflux for 23 hr. while passing a slow current of nitrogen through it. After cooling, it was separated into a phenolic (283 mg. of brown oil) and acidic (182 mg. of brown solid) constituents.

The phenolic fraction was chromatographed on 15 g. of Merck acid-washed alumina and elution with benzene–ether (98:2) furnished 92 mg. of 3-methoxy-4,5-methylenedioxyphenol (XIV), m.p. 86–87°, raised to 88–89° upon further recrystallization. The phenol gave a green color with ferric chloride, a purple Hansen[18] and a greenish-blue Lebat[19] test; $\lambda_{\max}^{\text{EtOH}}$ 287 mμ (log ε 3.59); $\lambda_{\max}^{\text{Nujol}}$ 2.87, 5.99, 6.12, 12.10, 12.35 and 12.50 μ.

Anal. Calcd. for $C_8H_8O_4$: C, 57.14; H, 4.80; O, 38.06; CH_3O, 18.45; mol. wt., 168. Found: C, 57.01; H, 4.78; O, 38.00; CH_3O, 18.62; mol. wt., 164 (by electrometric titration[44] in 66% dimethylformamide; pK_a 12.2). The acidic fraction was decolorized with charcoal and recrystallized twice from ether–hexane to yield 152 mg. of *o*-**methoxyphenylacetic acid (XIX)**, m.p. 119–121°, undepressed when mixed with a synthetic specimen.[16]

Anal. Calcd. for $C_9H_{10}O_3$: C, 65.95; H, 6.07; O, 28.88; CH_3O, 18.67. Found: C, 65.15; H, 6.27; O, 28.61; CH_3O, 18.53.

1,3-Dimethoxy-5,6-methylenedioxybenzene (VIII).—The above phenol (35 mg.) was heated under reflux for 3 hr. with 0.1 cc. of dimethyl sulfate and 1.0 g. of anhydrous potassium carbonate in 5 cc. of dry acetone. After filtering and evaporating the filtrate to dryness, the residue was passed in 1:1 benzene–hexane through 1.0 g. of Merck alumina and crystallized from ether–hexane at −10°; yield 19 mg., m.p. 38–40°. Sublimation at 60–70° and 3 mm. afforded the analytical sample, m.p. 40–40.5°, $\lambda_{\max}^{\text{EtOH}}$ 287 mμ (log ε 3.58), $\lambda_{\max}^{\text{CHCl}_3}$ 6.02 and 6.09 μ (both very strong)[45]; $\lambda_{\max}^{\text{KBr}}$ 6.05, 6.14, 12.60 and 12.75 μ.

Anal. Calcd. for $C_9H_{10}O_4$: C, 59.33; H, 5.53. Found: C, 59.57; H, 5.61.

Partial Demethylation of Tlatlancuayin (XII).—Tlatlancuayin (200 mg.) was heated under reflux for 14 hr. with a solution of 2.0 g. of anhydrous aluminum chloride in 12 cc. of absolute ether. The yellow solid, formed when the mixture was poured into ice-water, was collected and boiled for 20 min. with 20 cc. of acetic acid and 10 cc. of concd. hydrochloric acid. Dilution with water and extraction with ether furnished yellow crystals which were purified by chromatography on 8 g. of Merck acid-washed alumina. Elution with benzene and recrystallization from methylene chloride–hexane provided pale yellow plates (112 mg.) of **5-demethyltlatlancuayin (XXIV)**, m.p. 192–193°, green color with ferric chloride, $\lambda_{\max}^{\text{EtOH}}$ 243 and 270 mμ (log ε 4.27 and 4.29), $\lambda_{\text{inflect.}}^{\text{EtOH}}$ 335 mμ (log ε 3.61); $\lambda_{\max}^{\text{CHCl}_3}$ 5.92, 6.08 and 6.26 μ as well as "methylenedioxy bands" at 8.50, 8.86, 9.09, 9.30 and 9.49 μ.

Anal. Calcd. for $C_{17}H_{12}O_6$: C, 65.38; H, 3.87; CH_3O, 9.90. Found: C, 65.06; H, 3.91; CH_3O, 9.80.

Further evidence for the intact methylenedioxy grouping was presented by methylating 45 mg. of the above phenol XXIV with dimethyl sulfate in acetone solution in the presence of potassium carbonate (42 hr.) whereupon 42 mg. of tlatlancuayin (XII), m.p. 145–146.5°, was isolated.

In order to demonstrate that the methoxyl group at C-5 rather than at C-2′ had been cleaved, a mixture of 5-demethyltlatlancuayin (XXIV) was boiled for 16 hr. in a current of nitrogen with 8 g. of sodium hydroxide and 50 cc. of water. The acidic fraction (145 mg.) was decolorized with Norit and recrystallized, whereupon *o*-methoxyphenylacetic acid (XIX), m.p. and mixture m.p. 121–123°, was obtained.

Anal. Calcd. for $C_9H_{10}O_3$: C, 65.05; H, 6.07. Found: C, 65.34; H, 6.20.

Conversion of Tlatlancuayin (XII) to 2′,5,6,7-Tetrahydroxyisoflavone (XVIa).—Tlatlancuayin (500 mg.) was heated under a reflux for 8 hr. with 5.0 g. of aluminum chloride and 80 cc. of dry benzene, 3.0 g. of aluminum chloride was added and heating continued for an additional 12 hr. After concentrating to about 25 cc. and cooling, 20 cc. of acetic acid and 15 cc. of concd. hydrochloric acid were added and the mixture was kept at the reflux temperature for 30 min. A red precipitate formed which dissolved upon addition of water. Extraction with ether, washing until neutral, decolorization with Norit and evaporation to dryness led to 408 mg. of the yellow tetrahydroxyisoflavone XVIa, m.p. 193–202°. Recrystallization from hexane–acetone gave long yellow plates, m.p. 204–206°, brownish-green color with ferric chloride, $\lambda_{\max}^{\text{EtOH}}$ 273 mμ (log ε 4.20), $\lambda_{\text{inflect.}}^{\text{EtOH}}$ 235 and 320 mμ (log ε 4.15 and 3.72); $\lambda_{\max}^{\text{Nujol}}$ 3.03, 5.95, 6.09, 6.13 and 6.32 μ.[46] The substance gave negative Hansen and Lebat tests.

Anal. Calcd. for $C_{15}H_{10}O_6$: C, 62.94; H, 3.52; mol. wt., 286. Found: C, 62.97; H, 3.59; mol. wt. 275 ± 10 (by electrometric titration[44] in 66% dimethylformamide; pK_a 8.5 and 13.5).

2′,5,6,7-Tetramethoxyisoflavone (XVIb).—Methylation of 250 mg. of XVIa was accomplished by heating under reflux for 44 hr. with 30 cc. of dry acetone, 8.0 g. of anhydrous potassium carbonate and 1.2 cc. of dimethyl sulfate. After the usual processing including chromatography and recrystallization from methylene chloride–hexane there was obtained 206 mg. of colorless needles. The tetramethoxyisoflavone formed dimorphic crystals, needles (m.p. 117–122°) and plates (m.p. 148–149°) the transformation of which can be observed readily under a microscope, negative ferric chloride reaction, $\lambda_{\max}^{\text{EtOH}}$ 247 and 281 mμ (log ε 4.57 and 4.29), $\lambda_{\text{inflect.}}^{\text{EtOH}}$ 304 mμ (log ε 4.10), $\lambda_{\max}^{\text{CHCl}_3}$ 6.03 and 6.18 μ (both intense and sharp bands).

Anal. Calcd. for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30; O, 28.04; CH_3O, 36.02. Found: C, 66.64; H, 5.49; O, 28.20; CH_3O, 35.35.

***o*-Methoxybenzyl 2,3,4-Trimethoxy-6-hydroxyphenyl Ketone (XVIII).**—Cleavage of 150 mg. of the tetramethoxyisoflavone XVIb was carried out by boiling for 70 hr. in a slow current of nitrogen with 3.0 g. of potassium hydroxide and 30 cc. of water. The phenolic portion was filtered in benzene solution through 8 g. of Merck acid-washed alumina and recrystallized from ether–hexane to yield 132 mg. of colorless crystals, m.p. 63–65°, green ferric chloride reaction, $\lambda_{\max}^{\text{EtOH}}$ 280 and 332 mμ (log ε 4.34 and 3.81), $\lambda_{\max}^{\text{Nujol}}$ 6.08 and 6.19 μ.

Anal. Calcd. for $C_{18}H_{20}O_6$: C, 65.05; H, 6.07; O, 28.89. Found: C, 65.23; H, 5.98; O, 28.63.

Alkali Cleavage of *o*-Methoxybenzyl 2,3,4-Trimethoxy-6-hydroxyphenyl Ketone (XVIII).—The above desoxybenzoin derivative XVIII (110 mg.) was heated under reflux for 16 hr. in a current of nitrogen with 2.0 g. of potassium hydroxide and 10 cc. of water. The usual work-up led to 27 mg. of *o*-**methoxyphenylacetic acid (XIX)**, m.p. 121–123°, and 58 mg. of a phenolic fraction. The latter was treated with Norit and recrystallized from ether–hexane to afford 13 mg. of long colorless needles of **antiarol (XXI)**,[29] m.p. 145.5–146°. Identity with an authentic sample of antiarol, provided by Prof. W. Baker (University of Bristol), was established by mixture melting point determination and by infrared comparison; $\lambda_{\max}^{\text{Nujol}}$ 3.03, 6.16, 12.16 and 12.84 μ.

Anal. Calcd. for $C_9H_{12}O_4$: C, 58.69; H, 6.57. Found: C, 58.41; H, 6.67.

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(44) Measured in the Eli Lilly Research Laboratories, Indianapolis, Ind., through the courtesy of Dr. R. T. Rapala.

(45) On the other hand, 1,4-dimethoxy-2,3-methylenedioxybenzene (V) (ref. 21) exhibited only one intense band in that region at 6.15 μ.

(46) Synthetic 3′,4′-methylenedioxy-5,7-dihydroxyisoflavone[38] exhibited $\lambda_{\max}^{\text{Nujol}}$ 3.03, 6.01, 6.18 and 6.34 μ.