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CONSTITUENTS OF *CASIMIROA EDULIS* LLAVE *ET* LEX.—VI[1]

2',5,6-TRIMETHOXYFLAVONE, 2',5,6,7-TETRAMETHOXYFLAVONE (ZAPOTIN) AND 5-HYDROXY-2',6,7-TRIMETHOXYFLAVONE (ZAPOTININ)

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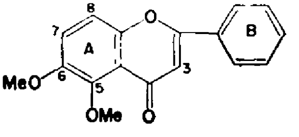
Abstract—Experiments are described which show that a substance isolated from the root bark of *Casimiroa edulis* Llave *et* Lex. is 2',5,6-trimethoxyflavone (IIa). It is demonstrated that zapotin and zapotinin, isolated previously from the same plant, are very probably 2',5,6,7-tetramethoxyflavone (IIIa) and 5-hydroxy-2',6,7-trimethoxyflavone (IIIb), respectively. The occurrence in nature of flavonoids of type IIa, IIIa and IIIb bearing a single oxygen substituent in ring B at the 2'-position is unusual.

2',5,6-*Trimethoxyflavone*

The isolation[2,3] and structure elucidation[4] of the carbostyril alkaloid casimiroin, a constituent of the fruit and bark of the tree *Casimiroa edulis* Llave *et* Lex., has been described previously. Pure casimiroin had been obtained by our group[3] by careful chromatography on alumina followed by crystallization to constant melting point. We have now found that the mother liquors remaining after this crystallization contain another substance.

Indications of this second product were first obtained when it was found that impure casimiroin derived from the root bark[3b] on being boiled with 20 per cent hydrochloric acid yielded the colourless casimiroinol[2,3a] accompanied by a yellow impurity. Casimiroin mother liquors were therefore re-chromatographed on alumina, when the acompanying substance was obtained as colourless plates, m.p. 125°.

The empirical formula of this compound was found to be $C_{18}H_{16}O_5$ and it contained three methoxyl groups. The spectral data (multiple absorption in the 6·0–6·5 μ region in the infra-red; ultra-violet maxima at 234, 268 and 328 mμ) were quite similar to those of 5,6-dimethoxyflavone (I) already found[3b] in the bark of *Casimiroa edulis*. These facts strongly suggested a trimethoxy-flavone or -isoflavone structure.[5]



I

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[1] Part V: F. Sondheimer, A. Meisels and F. A. Kincl, *J. Org. Chem.* **24**, 870 (1959).

[2] F. B. Power and T. Callan, *J. Chem. Soc.* **99**, 1993 (1911).

[3a] F. A. Kincl, J. Romo, G. Rosenkranz and F. Sondheimer, *J. Chem. Soc.* 4163 (1956); [b] J. Iriarte, F. A. Kincl, G. Rosenkranz and F. Sondheimer, *Ibid.* 4170 (1956).

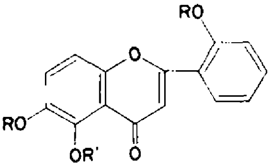
[4] A. Meisels and F. Sondheimer, *J. Amer. Chem. Soc.* **79**, 6328 (1957).

[5] The ultra-violet spectrum does not allow a clear distinction to be made between these classes of compounds, as is evident from an inspection of the spectra of a variety of flavones[6,12b] and isoflavones.[7]

The methoxyl groups could be cleaved by means of hydriodic acid in acetic anhydride, which yielded the corresponding yellow trihydroxy-compound, $C_{15}H_{10}O_5$, m.p. 280°, characterized as the triacetate, $C_{21}H_{16}O_8$, m.p. 142°. No rearrangement[8] had occurred in the demethylation reaction, since methylation of the product with methyl sulphate and potassium carbonate in acetone regenerated the starting material. Fusion of the trihydroxy-compound with potassium hydroxide at 210° yielded over 50 per cent of salicylic acid, but no other phenol except for a considerable amount of unchanged starting material could be isolated. The stability of the trihydroxy-compound to alkali is noteworthy and speaks against an isoflavone structure, since the latter class of compound is usually cleaved readily with base.[7,9] It appears therefore that the natural compound is a 2′-methoxyflavone or alternatively a flavone unsubstituted in ring A, either structure accounting for the formation of salicylic acid.

Boiling of the natural compound with 20 per cent hydrochloric acid resulted in the cleavage of only one methoxyl group and gave 80 per cent of a yellow dimethoxy-monohydroxy compound, m.p. 154°. This substance (which is the yellow impurity found in the conversion of crude casimiroin to casimiroinol, referred to above) was also obtained from the trihydroxy-compound by methylation with diazomethane. The ready demethylation of one methoxyl group and resistance to methylation of the corresponding hydroxyl group is typical of a substituent at the 5-position.[10a] That the hydroxyl group in the dimethoxy-hydroxy compound is at C-5 is confirmed by the fact that no hydroxyl band in the 3 μ region was apparent in its infra-red spectrum (due to chelation)[11] and that it gave a dark green colour with ferric chloride.[6b,12a] A ring A-unsubstituted structure is therefore excluded and two of the three methoxyl groups are at the 2′ and 5-positions. The third methoxyl group was tentatively placed at C-6 since the trihydroxy-compound gave a stable yellow-brown colour with sodium hydroxide, indicative of two vicinal hydroxyl groups.[12b]

Fortunately, 2′,5,6-trimethoxyflavone (IIa) has recently been synthesized by Doporto *et al.*[13] and the melting points reported for this substance as well as for the



II a, R=R′=Me
b, R=R′=H
c, R=R′=Ac
d, R=Me, R′=H

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[6] *Inter al.* [a] S. Aronoff, *J. Org. Chem.* **5**, 561 (1940); [b] L. H. Briggs and R. H. Locker, *J. Chem. Soc.* 3136 (1951).

[7] *Inter al.* [a] W. K. Warburton, *Quart. Rev.* **8**, 67 (1954); [b] P. Crabbé, P. R. Leeming and C. Djerassi, *J. Amer. Chem. Soc.* **80**, 5258 (1958); [c] L. H. Briggs and B. F. Cain, *Tetrahedron* **6**, 143 (1959).

[8] R. C. Shah, C. R. Mehta and T. S. Wheeler, *J. Chem. Soc.* 1555 (1938); W. Baker, N. C. Brown and J. A. Scott, *Ibid.* 1922 (1939); K. M. Gallagher, A. C. Hughes, M. O'Donnell, E. M. Philbin and T. S. Wheeler, *Ibid.* 3770 (1953).

[9] A. Robertson, C. W. Suckling and W. B. Whalley, *J. Chem. Soc.* 1571 (1949); F. E. King, T. J. King and A. J. Warwick, *Ibid.* 96 (1952).

[10] S. Wawzonek, *Heterocyclic Compounds* (Edited by R. C. Elderfield) Vol. 2; [a] p. 265; [b] p. 267; [c] p. 268. John Wiley, New York (1951).

[11] H. L. Hergert and E. F. Kurth, *J. Amer. Chem. Soc.* **75**, 1622 (1953); B. L. Shaw and T. H. Simpson, *J. Chem. Soc.* 655 (1955).

[12] T. A. Geissmann, *Modern Methods of Plant Analysis* (Edited by K. Paech and M. V. Tracey) [a] p. 467; [b] pp. 468–470; [c] pp. 471–472; [d] p. 487. Springer Verlag, Berlin (1955).

[13] M. L. Doporto, K. M. Gallagher, J. E. Gowan, A. C. Hughes, E. M. Philbin, T. Swain and T. S. Wheeler, *J. Chem. Soc.* 4249 (1955).

trihydroxy-compound IIb and the triacetate IIc agreed well with those of the corresponding compounds derived from *Casimiroa edulis*. Samples* of synthetic 2′,5,6-trimethoxyflavone (IIa) and 2′,5,6-trihydroxyflavone (IIb) were obtained and we prepared 5-hydroxy-2′,6-dimethoxyflavone (IId) from the latter by treatment with diazomethane. Direct comparison showed that the three synthetic substances were completely identical with the respective natural compounds, and our structural assignments are therefore correct.

Zapotin (2′,5,6,7-*tetramethoxyflavone*) *and zapotinin* (5-*hydroxy*-2′,6,7-*trimethoxy-flavone*)

We now turn to a consideration of the structures of zapotin and zapotinin, two further flavonoid constituents isolated from *Casimiroa edulis* Llave *et* Lex.[3] Zapotin, $C_{19}H_{18}O_6$, containing four methoxyl groups, was considered to be the tetramethyl ether of a tetrahydroxyflavone or isoflavone, although the exact structure was not elucidated.[3a] Zapotinin, $C_{18}H_{16}O_6$, containing three methoxyl and one hydroxyl group, was shown to be a corresponding trimethyl ether and it could be obtained from zapotin by fusion with potassium hydroxide at 270°.[3a]

Zapotin in its properties and reactions closely resembles the above-described 2′,5,6-trimethoxyflavone (IIa), including the infra-red and ultra-violet spectra.[3a] Boiling 20 per cent hydrochloric acid has now been found also to result in the cleavage of one methoxyl group to yield over 90 per cent of zapotinin.[14] Boiling hydriodic acid has been shown to convert zapotin to the corresponding tetrahydroxy-compound, demethylzapotin. Treatment of the latter substance with diazomethane leads to zapotinin, only three of the four hydroxyl groups being methylated.[3a] The fourth hydroxyl group is attacked when the methylation is carried out with methyl sulphate and potassium carbonate in acetone, the product being zapotin (see Experimental).

The stability of the ring system of zapotin and zapotinin to base again suggests a flavone rather than an isoflavone structure. The fact that one methoxyl group in zapotin is cleaved readily, while the corresponding hydroxyl group is methylated only with difficulty, is also indicative of a 5-methoxy structure.[10a] This is confirmed by the fact that zapotinin gives a dark green colour with ferric chloride[6b,12a] and in the infra-red does not show a hydroxyl band in the 3 μ region.[11]

Alkali fusion of demethylzapotin had previously given salicylic acid in high yield.[3a] Since a 5-hydroxyl group is present, this acid must be derived from ring B and consequently zapotin appears to be a 2′,5-oxygenated flavone with no other substituents in ring B. The alkali fusion of demethylzapotin had also given resorcinol in low yield as the only phenol to be isolated, this compound probably being formed by secondary changes. Repeated attempts to isolate other phenols were unsuccessful and no structural information regarding the oxygenation pattern of ring A could be obtained by this means.

The positions which can be considered for the remaining two methoxyl groups in zapotin are the 3-, 6-, 7-, and 8-positions. A 3-methoxy structure can be excluded since demethylzapotin does not give a bright colour with magnesium and hydrochloric

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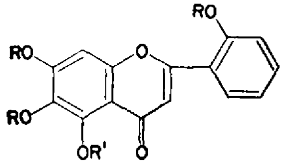
* Kindly provided by Professor T. S. Wheeler and Dr. E. M. Philbin.

[14] Zapotin in benzene solution is unaffected on treatment with 4% hydrochloric acid at room temperature, the conditions under which it was isolated.[3] It therefore appears that zapotinin is not an artefact formed from zapotin during the isolation process, although this possibility cannot be excluded with complete certainty.

acid (Shinoda test for flavonols).[12c] The 8-position is also apparently unsubstituted since demethylzapotin gives no colour with *p*-benzoquinone (a 5,8-dihydroxyflavone would have been oxidized to a quinone under these conditions).[15] Zapotin therefore appears to be 2′,5,6,7-tetramethoxyflavone (IIIa) and zapotinin 5-hydroxy-2′,6,7-trimethoxyflavone (IIIb). In agreement with these formulations is the fact that demethylzapotin (IIIc) with sodium hydroxide undergoes a series of colour changes to be expected if at least three hydroxyl groups are in adjacent positions [12b,16], and with sodium amalgam in ethanol it forms a green flocculent precipitate (this reaction, the Bargellini test, is indicative of a 5,6,7-trihydroxyflavone, although it does not definitely exclude certain other oxygenation patterns).[10c,17] The flavone structure is confirmed by the fact that zapotin and demethylzapotin show quite different physical properties from those of the recently described[7b] 2′,5,6,7-tetramethoxy- and 2′,5,6,7-tetrahydroxyisoflavone, respectively. There seems little doubt of the corrections of structure IIIa for zapotin and IIIb for zapotinin, and we hope to provide final proof through synthesis.



III *a*, R=R′=Me
b, R=Me, R′=H
c, R=R′=H

It is of interest to note from the biogenetic standpoint that 5,6-dimethoxyflavone (I), 2′,5,6-trimethoxyflavone (IIa) and 2′,5,6,7-tetramethoxyflavone (IIIa) occur together in the same plant. Of especial interest is the fact that 2′,5,6-trimethoxyflavone, zapotin and zapotinin appear to be the first examples of natural flavones (as opposed to flavonols) bearing a single oxygen substituent in ring *B* at the 2′-position. This substitution pattern in ring B is also quite rare in other naturally occurring flavonoids, only one example each in the flavonol,[18a] flavanone[18b] and isoflavone[18c] series being known.

EXPERIMENTAL[19]

Isolation of 2′,5,6-trimethoxyflavone (IIa) *from crude casimiroin*

Crude casimiroin (4·2 g; m.p. 198–199°; derived from the root bark of *Casimiroa edulis* Llave *et* Lex.[3b]) was crystallized from acetone–hexane. The dried mother liquors (1·0 g) were then chromatographed on 30 g alumina (Merck "acid-washed"). Benzene eluted 0·65 g 2′,5,6-trimethoxyflavone, m.p. 118–119°, which after crystallization from acetone formed colourless plates, m.p. 124–125° (reported:[18] m.p. 124–125°); infra-red bands at 6·06, 6·15 and 6·34 μ; λ_{max} 234, 268 and 328 mμ (log ε, 3·91, 4·02 and 3·88, respectively). An alcoholic solution with magnesium and hydrochloric acid gave an orange colour (3-methoxy group absent).[12c] (Found: C, 69·43; H, 4·93; CH_3O, 29·68. Calc. for $C_{18}H_{16}O_5$: C, 69·22; H, 5·16; 3 CH_3O, 29·78%).

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[15] The gossypitone reaction; see footnote 10*b* and A. G. Perkin, *J. Chem. Soc.* **103,** 657 (1913).

[16] L. R. Row and T. R. Seshadri, *Proc. Ind. Acad. Sci.* **22A,** 215 (1945).

[17] G. Bargellini, *Gazz. Chim. Ital.* **49,** II, 47 (1919); P. S. Rao and T. R. Seshadri, *Proc. Ind. Acad. Sci.* **17A,** 119 (1943).

[18a] Datiscetin (2′,5,7-trihydroxyflavonol): J. Kalff and R. Robinson, *J. Chem. Soc.* 1968 (1925); [b] citronetin (5,7-dihydroxy-2′-methoxyflavanone): *inter al.* T. H. Simpson and W. B. Whalley, *Ibid.* 166 (1955); [c] tlatlancuayin (2′,5-dimethoxy-6,7-methylenedioxyisoflavone): footnote 7*b*.

[19] Melting points are uncorrected. Ultra-violet spectra were measured in 95% ethanol solution on a Unicam S.P. 500 spectrophotometer and infra-red spectra in chloroform solution on a Baird double-beam recording spectrophotometer with sodium chloride optics. Analyses were carried out in our microanalytical department under the direction of Mr. Erich Meier.

2′,5,6-*Trihydroxyflavone* (IIb) *from* 2′,5,6-*trimethoxyflavone* (IIa)

A solution containing 83 mg of the trimethoxy-compound IIa, 1 cc acetic anhydride and 3 cc hydriodic acid (*d.* 1·7) was heated in an oil bath at 140° for 2 hr. The solution was cooled, treated with ice-water and the resulting precipitate (70 mg) was collected, washed with water and dried. Crystallization from acetone–chloroform gave 2′,5,6-trihydroxyflavone (48 mg) as yellow needles, m.p. 278–280° dec. (reported:[13] m.p. 274–278° dec.); λ_{max} 278 and 338 mμ (log ε, 4·65 and 4·37, respectively). It gave a stable yellow-brown colour with aqueous sodium hydroxide (two vicinal hydroxyl groups present).[12b] (Found: C, 66·37; H, 3·71; CH_3O, 0·00; act. H, 1·08. Calc. for $C_{15}H_{10}O_5$: C, 66·67; H, 3·73; 3 act. H, 1·12%).

2′,5,6-*Triacetoxyflavone* (IIc)

This substance was obtained from 2′,5,6-trihydroxyflavone (IIb) through boiling with sodium acetate in acetic anhydride for 1 hr. It formed colourless needles from methanol, m.p. 140–142° (reported:[13] m.p. 144–146°). (Found: C, 63·46; H, 3·84. Calc. for $C_{21}H_{16}O_8$: C, 63·63; H, 4·07%).

2′,5,6-*Trimethoxyflavone* (IIa) *from* 2′,5,6-*trihydroxyflavone* (IIb)

The trihydroxy-compound IIb (50 mg) was boiled under reflux for 6 hr with 5 g methyl sulphate and 10 g potassium carbonate in 30 cc dry acetone. Crystallization of the product from acetone gave 26 mg of the trimethoxy-compound IIa, m.p. 123–124°, undepressed on admixture with the natural compound.

5-*Hydroxy*-2′,6-*dimethoxyflavone* (IId)

(a) *From* 2′,5,6-*trimethoxyflavone* (IIa). The trimethoxy-compound IIa (100 mg) was boiled with 70 cc 20% hydrochloric acid for 30 min. The mixture was cooled, water was added and the yellow precipitate (90 mg) was collected, washed with water and dried. Crystallization from methanol gave 76 mg (80%) 5-hydroxy-2′,6-dimethoxyflavone as yellow needles, m.p. 153–154°; infra-red bands at 6·06, 6·18 and 6·33 μ, no hydroxyl band in the 2·7–3·1 μ region[11]; λ_{max} 272 and 329 mμ (log ε, 4·37 and 4·11, respectively). It gave a dark green colour with ethanolic ferric chloride (5-hydroxyl group present).[12a] (Found: C, 68·15; H, 4·81; CH_3O, 20·89; act. H, 0·38. $C_{17}H_{14}O_5$ requires: C, 68·45; H, 4·73; 2 CH_3O, 20·78; 1 act. H, 0·34%).

(b) *From* 2′,5,6-*trihydroxyflavone* (IIb). Treatment of the trihydroxy-compound IIb in methanol solution with excess ethereal diazomethane at 5° for 16 hr yielded over 90% of the 2′,6-dimethyl ether IId, m.p. 153–154°. There was no depression in m.p. on admixture with a sample prepared by method (a) and the infra-red spectra were identical.

Fusion of 2′,5,6-*trihydroxyflavone* (IIb) *with potassium hydroxide*

The powdered trihydroxy-compound IIb (130 mg) was added to 1·3 g potassium hydroxide and 0·2 cc water at 130°. The temperature was gradually increased to 170° during the next 30 min. More potassium hydroxide (0·3 g) was then added, the temperature raised to 210° during 15 min and kept there for a further 15 min. The clear brown melt was allowed to cool and water and dil hydrochloric acid were added. The product was extracted with ether and the ether layer was washed successively with sodium bicarbonate and sodium hydroxide solution. The bicarbonate extract was acidified with hydrochloric acid, extracted with ether and this ether solution dried and evaporated. Sublimation of the residue at 100° (25 mm) and subsequent crystallization from ether–hexane gave 35 mg (53%) salicyclic acid, m.p. 159–160°, identified with an authentic sample (m.p. 159–160°) through non-depression of the m.p. on admixture and the identity of the infra-red spectra.

The sodium hydroxide extract on acidification and extraction with ether gave 34 mg (26%) of the unchanged trihydroxy-compound IIb, but no other phenol could be identified.

Comparison of synthetic with natural 2′,5,6-*trioxygenated flavones*

Synthetic 2′,5,6-trimethoxyflavone (IIa) showed m.p. 122–124°, 2′,5,6-trihydroxyflavone (IIb) showed m.p. 275–278° dec., and treatment of the latter with diazomethane gave 5-hydroxy-2′,6-dimethoxyflavone (IId), m.p. 153–154°. There were no m.p. depressions when each of these substances were mixed with the corresponding natural derivative and the infra-red spectra in each case were identical.

Zapotinin (IIIb) *by treatment of zapotin* (IIIa) *with hydrochloric acid*

Zapotin[8] (200 mg) was boiled under reflux with 50 cc 20% hydrochloric acid for 1 hr. The substance soon dissolved, but a yellow precipitate then separated. Water was added and the precipitate was collected, dried and crystallized from methanol. The resulting zapotinin (175 mg; 91%) showed m.p. 223–224° and was identified with an authentic sample (m.p. 224–225°)[8a] by mixture m.p. determination and infra-red comparison. It gave a dark green colour with ethanolic ferric chloride; there was no hydroxyl band in the 2·7–3·1 μ region of the infra-red (5-hydroxyl group present).[12a,11]

Zapotin (IIIa) *by methylation of zapotinin* (IIIb)

Zapotinin (100 mg) was heated under reflux for 6 hr with methyl sulphate (10 g) and potassium carbonate (20 g) in dry acetone (60 cc). Crystallization of the product from methanol gave zapotin as colourless prisms, m.p. 149–151°, identified with an authentic sample (m.p. 150–151°)[8] in the usual way.

Demethylzapotin (IIIc)

This substance was obtained by treatment of zapotin with hydriodic acid and acetic anhydride as described previously.[8a] It showed m.p. 320–325° dec.: λ_{max} 228, 270, 343, 349 and 366 mμ (log ε, 4·34, 4·32, 3·66, 3·65 and 3·61, respectively). An ethanolic solution gave a yellow colour with magnesium and hydrochloric acid (no 3-hydroxyl group).[12c] Addition of *p*-benzoquinone to an ethanolic solution resulted in no appreciable change of colour (no 5,8-dihydroxyl group).[15] A solution in 10% sodium hydroxide underwent colour changes from yellow to red-orange to green-brown to grey-brown within 20 min (at least three adjacent hydroxyl groups present).[12b,16] An ethanolic solution formed a green flocculent precipitate when heated with sodium amalgam (5,6,7-trihydroxy group present).[10c,17]

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