# 804. *The Constituents of* Casimiroa edulis *Llave* et *Lex. Part I. The Seed.**

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The seeds of the Mexican tree *Casimiroa edulis* Llave *et* Lex. contain seven substances in addition to the previously isolated casimiroin, casimiroedine, casimirolid, a " yellow phenolic substance " (identified as 9-hydroxy-4-methoxyfurano[3,2-*g*]benzopyran-7-one) (II; R = OH), β-sitosterol β-D-glucoside, and β-sitosterol. Of these seven, two were identified as palmitamide and *N*-benzoyltyramine, whereas the other five appear to be new.

*Casimiroa edulis* Llave *et* Lex. (Rutaceae) is a tree growing in the temperate region of Mexico and Central America. The fruit, known as " Zapote blanco " (literally " the white fruit "), is commonly eaten in Mexico and has a pleasant flavour. Since Francisco Hernandez [1] claimed the fruit to induce sleep and the kernels to be a powerful poison, a variety of physiological actions have been attributed to the plant. The literature up to 1911 has been adequately reviewed by Power and Callan [2] who carried out the only extensive previous chemical investigation of these seeds and found no foundation for the reported physiological activities.

However, reports regarding the hypnotic, sedative, and hypotensive action of the seed persisted.[3] We now report a chemical re-investigation of the alcohol-soluble constituents of the seed. A study of the bark is reported in the following paper.

Power and Callan [2] isolated six substances from the fruit, namely, casimiroin ($C_{24}H_{20}O_8N_2$), casimiroedine ($C_{17}H_{24}O_5N_2$), casimirolid ($C_{24}H_{28}O_6$), a " yellow phenolic substance " ($C_{16}H_{12}O_6$), β-sitosterol β-D-glucoside (at the time called " ipuranol " and identified later [4]), and β-sitosterol.† We have been able to obtain all of these, but of the new compounds could confirm the empirical formula only of casimiroedine. In addition, we have found seven further compounds, of which five (zapotin, zapotinin, zapoterin, eduline, and zapotidine) appear to be new and two (palmitamide and *N*-benzoyltyramine) are known. In view of the extensively described physiological action of tyramine (one of the active principles of ergot), the *N*-benzoyl derivative of this substance is probably responsible for some of the ascribed activity of the seed.

The separation procedure employed consisted in evaporating the alcoholic extract of the kernels, adding dilute hydrochloric acid and extracting the mixture successively with solvents of increasing polarity, whereby " acid " extracts were obtained. The remaining aqueous layer was then basified with ammonia and again treated with the same solvents to give " alkaline " extracts. The material from each solution was chromatographed unless it crystallised directly. The results are summarised in the Table.

The " acid " hexane extract on chromatography on alumina yielded successively β-sitosterol and palmitamide each of which was identified by comparison with an authentic specimen. Although β-sitosterol has been isolated previously from many different natural sources, this appears to be the first case of palmitamide's being found in the plant kingdom.

Chromatography of the " acid " benzene extract led to zapotin, casimiroin, and *N*-benzoyltyramine. Zapotin, $C_{19}H_{18}O_6$, formed a picrate, perchlorate, and oxime; it contains four methoxyl groups which, combined with the ultraviolet absorption data and general colour reactions, indicated that it was a tetramethoxy-flavone or -*iso*flavone. The close similarity of the infrared spectra in the carbonyl region between zapotin (6·03, 6·22,

* Submitted in honour of the seventieth birthday of Sir Ian Heilbron, D.S.O., F.R.S.

† In addition, benzoic acid and a mixture of fatty acids were obtained. Bickern (*Arch. Pharm.*, 1903, **241**, 166) isolated two other compounds, the " gluco-alkaloid " casimirin and casimirol, that were obtained neither by Power and Callan nor by ourselves.

[1] Hernandez " Rerum medicarum Novae Hispaniae thesaurus, etc.," Rome, 1651, Lib. III, p. 89.

[2] Power and Callan, *J.*, 1911, **99**, 1993.

[3] *Inter al.*, de Lille, *Anales inst. biol.* (*Mex.*), 1934, **5**, 45; Mendez, *J. Amer. Inst. Homeopathy*, 1937, **30**, 271; Ramirez and Rivero, *Rev. mensual med. Mexico*, 1936, 9, I, No. 3; *Farmacopea Mexicana*, 6th edn., 1952, p. 423.

[4] Power and Salway, *J.*, 1913, 399.

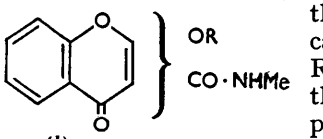
and 6·28 μ) and 5 : 6-dimethoxyflavone (6·03, 6·18, and 6·29 μ; see following paper) supports this formulation. With hydrogen iodide zapotin gave the tetrahydroxy-compound demethylzapotin, which on fusion with potassium hydroxide yielded salicylic acid and, as sole phenol, resorcinol (rather than the expected tri- or tetra-hydroxybenzene). Further work is being carried out to elucidate the structure of zapotin, which appears to differ from the known tetramethoxy-flavones and -*iso*flavones.

We consider our casimiroin to be identical with that of Power and Callan [2] in view of the similar properties of the bases and their aurichlorides and particularly since short treatment with hydrochloric acid gives an acid, m. p. 321—323° (reported: [2] m. p. >300°), which we name casimiroinol. Analysis of casimiroin points to the formula

| Compound | Extraction solvent | Yield (%) |
|---|---|---|
| β-Sitosterol | " Acid " hexane | 0·0027 |
| Palmitamide | ,, | 0·0003 |
| Zapotin | ,, benzene | 0·004 |
| Casimiroin (I; R = Me) | ,, | 0·0012 |
| *N*-Benzoyltyramine | ,, | 0·0015 |
| Furanocoumarin (II; R = OH) | ,, methylene chloride | 0·0112 |
| Zapotinin | ,, | 0·0005 |
| Zapoterin | ,, | 0·0027 |
| Casimirolid | ,, | 0·0021 |
| Sitosterol β-D-glucoside | ,, pentyl alcohol | 0·0016 |
| Eduline | " Alkaline " benzene | 0·0002 |
| Zapotidine | ,, methylene chloride | 0·0014 |
| Casimiroedine | ,, pentyl alcohol | 0·95 |

$C_{12}H_{11}O_4N$ rather than $C_{24}H_{20}O_8N_2$ proposed by Power and Callan.[2] On the basis of our formula, casimiroin contains one methoxyl and one *N*-methyl group and forms a monopicrate, but the aurichloride contains 2 mols. of casimiroin and one of aurichloric acid. It was apparently because of the last salt that the $C_{24}$ formula was favoured previously, since this requires the usual 1 : 1 aurichloride; direct molecular-weight determination of casimiroin points to the $C_{12}H_{11}O_4N$ formula. It has recently been shown [5] that salts of monosubstituted amides and hydrohalic acids may be formed in a 2 : 1 ratio and an elegant explanation was given: the same type of salt formation takes place between casimiroin and chloroauric acid.

Casimiroin was non-basic on titration with perchloric acid and this, combined with the infrared characteristics (see below), indicates an amide group. These facts suggest that the substance may be the methylamide of a methoxycoumarin-carboxylic or of a methoxychromonecarboxylic acid (cf. I; R = Me). Coumarins show an infrared band at *ca.* 5·80 μ [cf. the furanocoumarin (II; R = OH) and scopoletin (following paper)] due to the six-membered lactone grouping and the absence of this band in casimiroin rules out the coumarin structure. On the other hand, the striking similarity in the carbonyl region between 5 : 6-dimethoxyflavone (6·03, 6·18, and 6·29 μ; see following paper) and casimiroin (6·04, 6·12, 6·20, and 6·27 μ) strongly supports the chromone formulation (I; R = Me), the extra band at 6·12 μ being ascribed to the amide function.



(I)

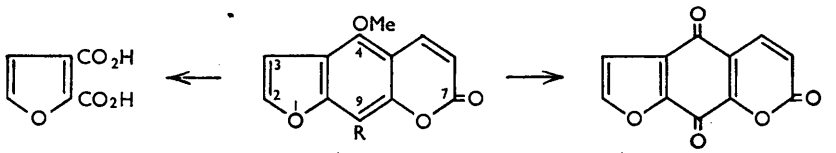
Casimiroinol has the formula $C_{11}H_9O_4N$. It is acidic (though showing no carboxyl function in the infrared spectrum) and contains no methoxyl group. Apparently it is related to casimiroin as a phenol to a methyl ether and accordingly treatment with diazomethane regenerated casimiroin.

The last compound to be eluted from the " acid " benzene extract had the formula $C_{15}H_{15}O_2N$. Ultraviolet absorption maxima at 226 (log ε 4·27) and 273 mμ (log ε 3·37) were due to a benzoate and a phenol group, respectively, as shown by the production of benzoic acid on alkaline fusion and disappearance of the 273 mμ band on acetylation. Methylation and subsequent oxidation furnished *p*-methoxybenzoic acid and the phenol was therefore *para*-substituted. The substance appeared to be *N*-benzoyltyramine (*N*-benzoyl-*p*-hydroxyphenethylamine), previously made by partial benzoylation of

[5] White, *J. Amer. Chem. Soc.*, 1955, **77**, 6215.

tyramine.[6] This was confirmed by preparation of the dibenzoyl derivative, which was identical with an authentic sample. Free tyramine, the decarboxylation product of tyrosine, has been found in ergot, mistletoe, and thistle.

The " acid " methylene chloride extract on direct crystallisation produced a yellow acidic substance, $C_{12}H_8O_5$, m. p. 223—224°, containing one methoxyl group. It is probably identical with the " yellow phenolic substance," m. p. 215—218°, of Power and Callan,[2] although these workers proposed the formula $C_{16}H_{12}O_6$. The presence of a hydroxyl function was shown by the formation of a monoacetate and a monobenzoate. Potassium permanganate oxidised it to furan-2 : 3-dicarboxylic acid (III), whereas chromium trioxide led to an orange product, $C_{11}H_4O_5$, containing no methoxyl group, which, it was suspected, was bergaptenquinone (IV).[7-9] The yellow substance, m. p. 223—224°, would then be 9-hydroxy-4-methoxyfurano[3,2-*g*]benzopyran-7-one (II; R = OH), obtained previously (m. p. 221°) from 4-aminobergapten (II; R = $NH_2$) by treatment with nitrous acid [10] and from byak-angelicin (II; R = $C_5H_{11}O_3$) [8] and phellopterin (II; R = $C_5H_9O$) [9] by acetic




acid–sulphuric acid. Confirmation was provided by the methylation of our furanocoumarin with diazomethane to the dimethyl ether, *iso*pimpinellin (II; R = OMe).[11] The alternative (unknown) 9-hydroxy-4-methoxy-formulation is unlikely in view of the good agreement of the physical properties of our substance and of its acetate with those reported [9] for the compound (II; R = OH). A number of 4- and/or 9-substituted furanocoumarins (II) have been found in Nature, but the 4-hydroxy-9-methoxy-compound (II; R = OH) has previously been obtained only by the aforementioned conversions.

The " acid " methylene chloride extract, from which the yellow furanocoumarin (II; R = OH) had been removed, was chromatographed and thereby yielded successively zapotinin, zapoterin, and casimirolid. The first of these, zapotinin, was a yellow substance, $C_{18}H_{16}O_6$, m. p. 223—225°, containing one hydroxyl group (monoacetate) and three methoxyl groups. The formal relation with zapotin ($C_{19}H_{18}O_6$; no hydroxyl and four methoxyl groups) was at once apparent. In fact, zapotinin was obtained from zapotin by treatment with potassium hydroxide and from demethylzapotin by diazomethane. Therefore zapotinin is a trimethyl ether of the tetrahydroxy-flavone or -*iso*flavone of which zapotin is the tetramethyl ether.

Zapoterin, $C_{19}H_{24}O_6$, had no high-intensity ultraviolet absorption, whereas the infrared spectrum showed several bands in the carbonyl region. On attempted acetylation no acetyl groups were introduced, but an isomer, *iso*zapoterin, was formed.

The last substance to be eluted from the chromatogram of the " acid " methylene chloride extract had m. p. 229—231°, $[\alpha]_D$ −49°, and is therefore almost certainly identical with Power and Callan's casimirolid [2] (m. p. 229—230°, $[\alpha]_D$ −49°). Analysis of our casimirolid, however, indicated the formula $C_{28}H_{32}O_8$ and not $C_{24}H_{28}O_6$ as proposed by the British workers. The relation between casimirolid and obacunone,[12] and the formula of the latter, will be discussed in a later paper.

The " acid " pentyl alcohol extract crystallised directly and yielded β-sitosterol β-D-glucoside, further characterised as the tetra-acetate.

[6] Barger, *J.*, 1909, **95**, 1123.
[7] Thomas and Baetcke, *Ber.*, 1912, **45**, 3705.
[8] Noguchi (Noguti) and Kawanami, *ibid.*, 1938, **71**, 344.
[9] *Idem*, *J. Pharm. Soc. Japan*, 1940, **60**, 57.
[10] *Idem*, *Ber.*, 1938, **71**, 1428.
[11] *Inter al.*, Wessely and Kallab, *Monatsh.*, 1932, **59**, 161; Caldwell and Jones, *J.*, 1945, 540.
[12] Kaku and Ri, *J. Pharm. Soc. Japan*, 1935, **55**, 222; Emerson, *J. Amer. Chem. Soc.*, 1951, **73**, 2621.

The " alkaline " benzene extract on chromatography yielded eduline, m. p. 187—188°, a new alkaloid. Eduline has the composition $C_{17}H_{15}O_2N$, contains one methoxyl group, and forms a monopicrate and a monoperchlorate.

The " alkaline " methylene chloride extract produced a substance, $C_7H_9N_3S$, which we have named zapotidine. It contains one *N*-methyl group. When it is boiled with aqueous potassium hydroxide, acidification gives hydrogen sulphide and a basic liquid base with a smell reminiscent of pyridine. Zapotidine therefore appears to be a methyl-pyridyl-thiourea.

Finally, the " alkaline " pentyl alcohol extract furnished Power and Callan's casimiroedine,[2] $C_{17}H_{24}O_5N_2$, the constitution of which is being investigated by Professor Carl Djerassi.

Work is continuing on the substances for which we have suggested partial structures.

## Experimental

M. p.s were taken on a Kofler block. Rotations were determined (at 20°) in $CHCl_3$, and ultraviolet absorption spectra in 95% EtOH solution, unless otherwise specified. Thanks are offered to Miss M. T. Cardenas and Mrs. P. Lopez for these measurements and to Professor Carl Djerassi for providing the infrared spectra, which were taken with a Baird double-beam infrared recording spectrophotometer [only bands in the carbonyl region (5·5—6·4 μ) are given; (w) = weak]. Analyses were by Mrs. A. Gonzalez of Syntex, S.A., Mr. J. F. Alicino, Metuchen, N. J., Drs. G. Weiler and F. B. Strauss, Oxford, Dr. A. Bernhardt, Mülheim (Ruhr), and Dr. R. Dietrich, Zürich. Molecular weights were determined by the Rast method, and neutral equivalent determinations by perchloric acid titration.

*General Extraction Procedure.*—The fruit of *Casimiroa edulis* Llave *et* Lex. was collected in the State of Hidalgo, Mexico, during March. The fleshy pulp was separated from the kernels and the latter were dried in the sun. The dried kernels (100 kg.) were coarsely ground and extracted twice with hot ethanol (2 × 400 l.). The combined alcoholic extracts were evaporated and the resulting dark brown syrup was diluted with 4% hydrochloric acid (50 l.). The mixture was extracted successively with hexane, benzene, methylene chloride, and pentyl alcohol (5 × 10 l. each), to give the " acid " extracts.

The residual aqueous phase was made alkaline with concentrated aqueous ammonia, with ice-cooling, then extracted with benzene, methylene chloride, and pentyl alcohol (5 × 10 l. each), giving the " alkaline " extracts.

Each of the extracts was evaporated and the residue (1 part) was chromatographed on alumina (*ca.* 40 parts) in the usual way. Results are given in the Table.

β-*Sitosterol.*—β-Sitosterol was obtained from the " acid " hexane extract and was eluted from the column with benzene–ether (7 : 3). It formed plates (from methanol), m. p. 138—139°, $[\alpha]_D$ −38° (lit.,[13] m. p. 140°, $[\alpha]_D$ −37°). The m. p. was undepressed on admixture with an authentic sample and the infrared spectra were identical. The acetate had m. p. 127—128°, $[\alpha]_D$ −38° (Found: C, 81·45; H, 11·45. Calc. for $C_{31}H_{52}O_2$: C, 81·5; 11·5%) (lit.,[13] m. p. 127—128°, $[\alpha]_D$ −41°). The benzoate had m. p. 145—147°, $[\alpha]_D$ −12° (lit.,[13]: m. p. 146—147°, $[\alpha]_D$ −14° [13]).

*Palmitamide.*—This was obtained from the " acid " hexane extract, and was eluted from the column with benzene–ether (1 : 1). It crystallised from methanol as plates, m. p. 103—104° (Found: C, 75·15; H, 12·95; N, 5·5%; *M*, 224. Calc. for $C_{16}H_{33}ON$: C, 75·25; H, 13·0; N, 5·5%; *M*, 255). Identity with an authentic sample (prepared from palmitoyl chloride and ammonia), m. p. 104°, was established by a mixed m. p. determination and infrared comparison.

*Zapotin.*—The first substance obtained by chromatography of the " acid " benzene extract was *zapotin*, eluted with benzene–ether (4 : 1). It separated from methanol or acetone as colourless prisms, m. p. 150—151°, $\lambda_{max.}$ 230, 255, and 324 mμ (log ε 4·45, 4·20, and 3·83, respectively), $\lambda_{max.}$ (in $CHCl_3$) 6·03, 6·22, and 6·28 μ (w) (Found: C, 66·65; H, 5·45; OMe, 36·8%; *M*, 340. $C_{19}H_{18}O_6$ requires C, 66·65; H, 5·3; 4OMe, 36·25%; *M*, 342), and gave positive Wilson and Shinoda colour reactions for flavonoids and with concentrated sulphuric acid an intense yellow colour. The *picrate* formed yellow needles (from methanol), m. p. 181—182° (Found: C, 52·2; H, 3·9; N, 7·05. $C_{19}H_{18}O_6,C_6H_3O_7N_3$ requires C, 52·55; H, 3·7; N, 7·35%). The *perchlorate*, prepared in acetone, crystallised from acetone–ether as yellow needles,

[13] Elsevier, " Encyclopaedia of Organic Chemistry," 1940, Vol. XIV, p. 90.

m. p. 204—206° (Found: C, 51·2; H, 4·55; Cl, 8·2. $C_{19}H_{18}O_6,HClO_4$ requires C, 51·55; H, 4·3; Cl, 8·0%). The *oxime*, prepared in boiling methanol–pyridine for 2 hr., separated from ethyl acetate–ether as light yellow crystals, m. p. 240—243° (Found: C, 64·3; H, 5·25; N, 4·25. $C_{19}H_{19}O_6N$ requires C, 63·85; H, 5·35; N, 3·9%).

*Demethylzapotin.*—Zapotin (4 g.) in acetic anhydride (60 c.c.) and hydriodic acid (85 c.c.; *d* 1·7) was boiled under reflux for 1 hr. The cooled mixture was poured into ice and water, and the precipitate was washed with water and dried. This material (3·1 g.; m. p. 246—252°) on three successive sublimations in a high vacuum, yielded *demethylzapotin* as yellow needles, m. p. 321—325°, $\lambda_{max.}$ (mull) 5·96, 6·08, and 6·21 μ (Found: C, 63·2; H, 3·5; OMe, 0·0. $C_{15}H_{10}O_6$ requires C, 62·95; H, 3·5%). The substance gave a strong green colour with alcoholic ferric chloride.

*Salicylic Acid and Resorcinol by Alkali-fusion of Demethylzapotin.*—Powdered demethylzapotin (1 g.) was added gradually to potassium hydroxide (10 g.) and water (1 c.c.) at 130°. The temperature was increased to 170° during the next 30 min. and more potassium hydroxide (2 g.) was added. The temperature was raised to 210° during 15 min. and kept thereat for 10 min. The brown melt was allowed to cool, dissolved in water, and acidified with hydrochloric acid. The products were extracted with ether (*A*) and the organic extract was washed with sodium hydrogen carbonate solution. The aqueous layer was acidified and extracted with ether and this ether solution on drying, evaporation, and crystallisation from water produced salicylic acid (0·25 g.), m. p. and mixed m. p. 156—158° (identical infrared spectra).

The ether extract (*A*) was dried and evaporated. The resulting oil (0·22 g.), on benzoylation (Schotten–Baumann), yielded resorcinol dibenzoate (0·11 g.), m. p. and mixed m. p. 116·5° (from ethanol) (Found: C, 75·3; H, 4·7; O, 20·1. Calc. for $C_{20}H_{14}O_4$: C, 75·45; H, 4·45; O, 20·1%).

*Casimiroin* (I; R = Me).—*Casimiroin* was the second substance obtained by chromatography of the " acid " benzene extract and was eluted from the column with benzene–ether (3 : 1). It separated from methanol as feathery needles, m. p. 199—200°. Sublimation in a high vacuum yielded a sample, m. p. 202—203°, $\lambda_{max.}$ 226, 236, 252, 260, and 300 mμ (log ε 4·50, 4·45, 4·35, 4·34, and 3·84, respectively), $\lambda_{max.}$ (mull) 6·04, 6·12, 6·20, and 6·27 μ (Found: C, 61·8, 61·7; H, 4·7, 4·75; O, 27·55; N, 6·0; OMe, 13·4; *N*-Me, 6·1; *C*-Me, 0·0%; *M*, 214, 217. $C_{12}H_{11}O_4N$ requires C, 61·8; H, 4·75; O, 27·45; N, 6·0; 1OMe, 13·3; 1*N*-Me, 6·45%; *M*, 233) (Power and Callan [2] give m. p. 196—197°). Casimiroin gave an intense orange colour with tetranitromethane and a red-brown colour with concentrated nitric acid. It was recovered unchanged from acetic anhydride–pyridine at 90°. Casimiroin *picrate* crystallised from methanol as yellow needles, m. p. 193—194° (Found: C, 46·75; H, 3·35. $C_{12}H_{11}O_4N,C_6H_3O_7N_3$ requires C, 46·75; H, 3·05%) (Power and Callan [2] give m. p. 165°). Casimiroin *aurichloride*, prepared in aqueous ethanol, formed pale orange needles (from methanol), m. p. 196—198° [Found: C, 35·4, 35·65; H, 3·0, 3·1; N, 3·45; Au, 24·45; Cl, 17·65. $(C_{12}H_{11}O_4N)_2,HAuCl_4$ requires C, 35·75; H, 2·85; N, 3·45; Au, 24·45; Cl, 17·6%] (Power and Callan [2] give m. p. 195—196°).

*Casimiroinol* (I; R = H).—Casimiroin (0·3 g.) was boiled under reflux with 20% hydrochloric acid (15 c.c.). The compound dissolved, but after 20 min. a copious precipitate had separated. The mixture was cooled, and the solid washed with water and dried. Crystallisation from methanol–chloroform produced *casimiroinol* (0·26 g.) as needles, m. p. 321—323°, $\lambda_{max.}$ 230, 252, and 302 mμ (log ε 4·50, 4·35, and 3·90, respectively), $\lambda_{max.}$ (mull) 6·02 μ, 6·13, 6·20, and 6·40 μ [Found: C, 59·8; H, 4·5; O, 29·25; N, 6·2%; OMe, 0·0; equiv. (KOH), 229. $C_{11}H_9O_4N$ requires C, 60·25; H, 4·15; O, 29·2; N, 6·4%; equiv., 219]. This dissolved in dilute aqueous sodium hydroxide and was precipitated on acidification. Treatment in ether with diazomethane at 5° for 24 hr. regenerated casimiroin almost quantitatively.

N-*Benzoyltyramine.*—This substance was obtained from the " acid " benzene extract and was eluted from the column with ether–ethyl acetate (4 : 1). It crystallised from ether as plates, m. p. 161—162°, $\lambda_{max.}$ 226 and 273 mμ (log ε 4·27 and 3·37, respectively), $\lambda_{max.}$ (mull) 6·08, 6·21 (w), and 6·32 μ (w) (Found: C, 74·85; H, 6·15; N, 5·95%; *M*, 246. Calc. for $C_{15}H_{15}O_2N$: C, 74·65; H, 6·25; N, 5·8%; *M*, 241) (lit.,[6] m. p. 162°). The *acetate* (acetic anhydride–pyridine; 1 hr.; 90°) formed needles (from acetone–hexane), m. p. 121—122°, $\lambda_{max.}$ 222 mμ (log ε 4·15), $\lambda_{max.}$ (in $CHCl_3$) 5·76, 6·09, 6·26 (w), and 6·36 μ (w) (Found: C, 71·85; H, 6·4; N, 4·85; Ac, 15·5. $C_{17}H_{17}O_3N$ requires C, 72·05; H, 6·05; N, 4·95; Ac, 15·2%). The dibenzoyl derivative was prepared by means of benzoyl chloride and pyridine (1 hr., 90°): chromatography on alumina and crystallisation from acetone gave the derivative, m. p. and mixed m. p. 172—173° (identical infrared spectra).

Fusion of the monobenzoyl derivative with potassium hydroxide and a little water at 280° produced benzoic acid (m. p. and mixed m. p.). Methylation of it with diazomethane produced the methyl ether as plates, m. p. 123—124°, which on oxidation with alkaline potassium permanganate in water yielded *p*-methoxybenzoic acid (m. p. and mixed m. p.).

*9-Hydroxy-4-methoxyfurano*[3,2-g]*benzopyran-7-one* (II; R = OH).—This furanocoumarin was isolated from the " acid " methylene chloride extract and crystallised from methanol as yellow needles, m. p. 223—224°, $\lambda_{max.}$ 222, 272, and 314 mμ (log ε 4·47, 4·34, and 4·07, respectively), $\lambda_{max.}$ (mull) 5·80, 6·02 (w), 6·12 (w), and 6·20 μ (Found: C, 62·15; H, 3·4; OMe, 13·25%; *M*, 239. Calc. for $C_{12}H_8O_5$: C, 62·05; H, 3·45; 1OMe, 13·35%; *M*, 232] [reported by Power and Callan[2] for the " yellow phenolic substance," m. p. 215—218°; found: C, 63·6; H, 3·9; reported[9] for (II; R = OH), m. p. 221°]. The furanocoumarin dissolved in aqueous sodium hydroxide to a yellow solution. With concentrated sulphuric acid a red colour and with ferric chloride a yellow colour were produced. The acetate, prepared by use of pyridine and acetic anhydride (1 hr.; 90°), separated from methanol as plates, m. p. 181—182°, $\lambda_{max.}$ 221, 246, 266, and 308 mμ (log ε 4·38, 4·22, 4·24, and 4·13, respectively) (Found: C, 61·7; H, 3·8. Calc. for $C_{14}H_{10}O_6$: C, 61·3; H, 3·65%) (lit.,[9] m. p. 180°). The *benzoate*, obtained by means of benzoyl chloride and pyridine (10 min., 90°), formed prisms, m. p. 203—205°, from chloroform–ether (Found: C, 68·15; H, 3·35. $C_{19}H_{12}O_6$ requires C, 67·85; H, 3·6%).

iso*Pimpinellin* (II; R = OMe).—A solution of the furanocoumarin (II; R = OH) (200 mg.) in methanol (10 c.c.) was treated with ethereal diazomethane at room temperature. Crystallisation from acetone–ether gave *iso*pimpinellin (185 mg.) as light yellow needles, m. p. 150—151°, $\lambda_{max.}$ 222, 240, 248, 268, and 311 mμ (log ε 4·40, 4·13, 4·20, 4·32, and 4·14, respectively), $\lambda_{max.}$ (KBr disc) 5·80 and 6·21 μ (Found: C, 63·75; H, 4·2. Calc. for $C_{13}H_{10}O_5$: C, 63·4; H, 4·1%). An authentic specimen of *iso*pimpinellin (m. p. 150—151°) showed identical ultraviolet and infrared spectra and no depression in m. p. was observed on admixture.

*Bergaptenquinone* (IV).—Chromium trioxide (2·4 g.) in water (10 c.c.) was added to a solution of the furanocoumarin (II; R = OH) (3 g.) in glacial acetic acid (300 c.c.). After 30 min. at room temperature, orange crystals had appeared. The mixture was diluted with water, and the precipitate was collected, washed with water, dried, and crystallised from acetone. The resulting bergaptenquinone (2·14 g.) formed orange plates, m. p. 251—253° (decomp.), $\lambda_{max.}$ 273, 314, and 426 mμ (log ε 4·09, 3·80, and 3·28, respectively), $\lambda_{max.}$ (mull) 5·78, 6·02, 6·21 (w), and 6·40 μ (w) (Found: C, 61·5; H, 2·05; OMe, 0·0; *M*, 195. Calc. for $C_{11}H_4O_5$: C, 61·1; H, 1·85%; *M*, 216] (lit.,[7] m. p. 248—250°).

*Furan-2*: *3-dicarboxylic Acid* (III).—Potassium permanganate (10 g.) in water (100 c.c.) was added to a stirred solution of the furanocoumarin (II; R = OH) (3 g.) in 5% aqueous sodium hydroxide (100 c.c.) during 30 min. at room temperature. After a further 10 min., the precipitate was removed, then washed with water, and the filtrate after acidification extracted with ethyl acetate. Evaporation and crystallisation from acetone–ether gave furan-2: 3-dicarboxylic acid (0·29 g.), m. p. 220—221°, $\lambda_{max}$ 263 mμ (log ε 4·14) (Found: C, 46·5; H, 2·75. Calc. for $C_6H_4O_5$: C, 46·2; H, 2.6%) (lit.,[8] m. p. 221°).

*Zapotinin.*—(a) *From the seed.* This substance was isolated from the " acid " methylene chloride extract by chromatography on alumina of the mother-liquors obtained after removal of the furanocoumarin (II; R = OH). *Zapotinin* was eluted from the column with benzene and crystallised from methanol as yellow needles, m. p. 224—225°, $\lambda_{max.}$ 230, 264, and 345 mμ (log ε 4·34, 4·27, and 3·57, respectively), $\lambda_{max.}$ (mull) 5·96, 6·08, and 6·22 μ (Found: C, 65·95; H, 4·95; OMe, 28·55. $C_{18}H_{16}O_6$ requires C, 65·85; H, 4·9; 3OMe, 28·35%). Zapotinin gave an orange colour with concentrated sulphuric acid and a dark green colour with alcoholic ferric chloride. The *acetate* crystallised from ether–methanol as colourless needles, m. p. 214—216°, $\lambda_{max.}$ 226 and 320 mμ (log ε 4·42 and 3·87, respectively) (Found: C, 64·55; H, 4·85. $C_{20}H_{18}O_7$ requires C, 64·85; H, 4·9%).

(b) *From zapotin.* A mixture of zapotin (0·5 g.), potassium hydroxide (5 g.), and water (0·5 c.c.) was heated rapidly to 270° and kept at this temperature for 20 min. The mass was allowed to cool, then dissolved in water, and the solution was acidified with hydrochloric acid. The resulting precipitate on crystallisation from methanol gave zapotinin (0·41 g.), m. p. 223—225°. Identity with the substance isolated from the plant was established by mixed m. p. determination and infrared comparison.

(c) *From demethylzapotin.* Demethylzapotin (50 mg.) in methanol (3 c.c.) was methylated with ethereal diazomethane overnight at room temperature. Crystallisation from methanol gave zapotinin (12 mg.), m. p. 220—222°, undepressed on admixture with the naturally occurring material.

*Zapoterin.*—This compound was eluted from the column with benzene–methylene chloride (1 : 1) directly after zapotinin. *Zapoterin* formed prisms (from methanol), m. p. 257—259°, $[\alpha]_D$ −51°, no high-intensity ultraviolet absorption maximum, $\lambda_{max.}$ (KBr disc) 5·72, 5·85, 6·01, 6·16, and 6·36 μ (w) (Found : C, 65·75; H, 6·9; O, 27·0%; OMe, 0·0; *M*, 327. $C_{19}H_{24}O_6$ requires C, 65·5; H, 6·95; O, 27·55%; *M*, 348). The Molisch test for sugars was negative. Zapoterin gave a brown colour with concentrated sulphuric acid and a deep yellow colour with concentrated nitric acid. Zapoterin with acetic anhydride and pyridine at 90° for 1 hr. gave iso*zapoterin*, needles (from methanol), m. p. 284—285°, $\lambda_{max.}$ (KBr disc) 5·78, 5·86, and 6·12 μ (w) (Found : C, 65·4; H, 6·9; Ac, 0·0. $C_{19}H_{24}O_6$ requires C, 65·5; H, 6·95%.)

*Casimirolid.*—The chromatogram from which zapotinin and zapoterin had been removed was washed with methylene chloride. This resulted in the elution of casimirolid, prisms (from ethanol), m. p. 229—231°, $[\alpha]_D$ −49°, no high-intensity ultraviolet absorption maximum, $\lambda_{max.}$ (in $CHCl_3$), 5·78, 5·92, 6·12 (w), and 6·28 μ (w) (Found : C, 67·8; H, 6·5; O, 25·9%; *M*, 487. Calc. for $C_{28}H_{32}O_8$ : C, 67·7; H, 6·5; O, 25·8%; *M*, 496). (Power and Callan [2] give m. p. 229—230°, $[\alpha]_D$ −49°; found : C, 69·5; H, 6·9%; *M*, 426.)

β-*Sitosterol* β-D-*Glucoside.*—This substance was obtained from the " acid " pentyl alcohol extract. It crystallised directly from dioxan and had m. p. 290—295° (decomp.) (Found : C, 72·75; H, 10·25. Calc. for $C_{35}H_{60}O_6$ : C, 72·85; H, 10·5%) (lit.,[14] m. p. 285—290°). The tetra-acetate had m. p. 164—166° (lit.,[14] m. p. 166—168°).

*Eduline.*—The " alkaline " benzene extract on chromatography, elution with benzene–ether (9 : 1), and crystallisation from acetone–ether gave *eduline* as plates, m. p. 187—188°, $\lambda_{max.}$ 256, 336, and 350 mμ (log ε 4·53, 4·04, and 4·06, respectively), $\lambda_{max.}$ (in $CHCl_3$) 6·18 (w), 6·27, and 6·40 μ (Found : C, 76·95; H, 5·8; N, 5·2; OMe, 11·5. $C_{17}H_{15}O_2N$ requires C, 76·95; H, 5·7; N, 5·3; 1OMe, 11·7%). Eduline gave a red colour with tetranitromethane and a violet colour with concentrated nitric acid. The *picrate* separated from methanol as golden-yellow needles, m. p. 225—227° (decomp.) (Found : C, 56·2; H, 3·7; N, 11·5. $C_{17}H_{15}O_2N,C_6H_3O_7N_3$ requires C, 55·85; H, 3·65; N, 11·35%). The *perchlorate*, prepared in acetone, crystallised from acetone–ether as needles, m. p. 250—252° (Found : C, 55·65; H, 4·45; N, 3·9. $C_{17}H_{15}O_2N,HClO_4$ requires C, 55·8; H, 4·4; N, 3·85%).

*Zapotidine.*—The " alkaline " methylene chloride extract on chromatography, elution with benzene, and crystallisation from ether furnished *zapotidine* as plates, m. p. 96—98°, $\lambda_{max.}$ 236, 256, and 290 mμ (log ε 3·89, 3·91, and 3·87, respectively), $\lambda_{max.}$ (mull) 6·04 (w), 6·13, and 6·27 μ (Found : C, 50·5; H, 5·4; N, 25·15; S, 19·4; *N*-$CH_3$, 6·85%; *M*, 170; equiv., 167. $C_7H_9N_3S$ requires C, 50·3; H, 5·45; N, 25·15; S, 19·15; 1*N*-Me, 9·0%; *M*, 167). The *picrate* separated from methanol as yellow laths, m. p. 195—196° (Found : C, 39·35; H, 3·1; N, 21·2. $C_7H_9N_3S,C_6H_3O_7N_3$ requires C, 39·4; H, 3·05; N, 21·2%).

*Casimiroedine.*—Casimiroedine was obtained from the " alkaline " pentyl alcohol extract by direct crystallisation from pentyl alcohol or methanol as needles, m. p. 224—225°, $[\alpha]_D$ −33° (aq. 1% HCl), $\lambda_{max.}$ 218 and 280 mμ (log ε 4·12 and 4·24 respectively) (Found : C, 60·6; H, 7·05; N, 8·25. Calc. for $C_{17}H_{24}O_5N_2$ : C, 60·7; H, 7·2; N, 8·35%) {lit.,[2] m. p. 222—223°, $[\alpha]_D$ −36·5° (aq. 1% HCl)}.

The authors thank Messrs. Ernesto Visconti, Hector Macias, and A. Romo of the Instituto de Quimica de la Universidad Nacional Autonoma de Mexico for technical assistance, Mr. Santiago Fuentes of the Botanical Dept., Syntex, S.A., for the collection of the plant material, Professor F. Wessely of the University of Vienna for a sample of *iso*pimpinellin, and Dr. H. S. Vishniac of Haskins Laboratories, New York, for a sample of β-sitosterol.

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[*Received, April 10th*, 1956.]

[14] *Op. cit.*, Suppl., 1954, Vol. XIV, 1820 S, 1822 S.