thiamin. The insoluble material was recrystallized from hot water to yield 4.0 g. (80%) of VII, m.p. 217°.

(b) **By Reduction of Oxythiamin Chloride VI.**—Oxythiamin chloride, reduced with sodium borohydride by the method used above on thiamin, gave VII which melted at 217° and did not depress the melting point of a sample prepared by method a. The infrared spectra of the two samples were identical.

Anal. Calcd. for $C_{12}H_{19}N_3O_2S$: C, 53.53; H, 7.06. Found from (a): C, 53.56; H, 6.82. From (b): C, 53.54; H, 6.75.

Sulfite Cleavage of Tetrahydroöxythiamin (VII).—To 2.0 g. of tetrahydroöxythiamin dissolved in 3 ml. of concentrated hydrochloric acid, 10 ml. of water was added, followed by 4.0 g. of sodium bisulfite. The solution was adjusted to *p*H 5–5.5 with 5% sodium hydroxide. After standing for 2 days in a stoppered flask, the solution was made alkaline and extracted with chloroform. Treatment of the aqueous layer according to the method used[16] in isolating the oxysulfonic acid obtained from 2-methyl-4-oxy-5-ethoxymethylpyrimidine gave 1.2 g. (80%) of **2-methyl-4-oxypyrimidyl-5-methanesulfonic acid (VIII)**, m.p. 325°.

Anal. Calcd. for $C_6H_8N_2O_4S$: C, 35.29; H, 3.29; N, 13.72. Found: C, 35.10; H, 3.72; N, 13.40.

Dihydrothiamin (IX).—The reaction filtrate obtained in the above preparation of tetrahydrothiamin was saturated with solid sodium carbonate and extracted several times with chloroform. The combined extracts were evaporated and the residue leached with water. The insoluble tetrahydrothiamin was collected and recrystallized from hot water. The aqueous filtrate was saturated with solid sodium carbonate and extracted several times with chloroform. The residue obtained by reduced-pressure evaporation of the dried (over potassium carbonate) extract was recrystallized from absolute alcohol–petroleum ether (b.p. 30–60°) to yield IX, m.p. 175°.

Anal. Calcd. for $C_{12}H_{18}N_4OS$: C, 54.11; H, 6.82; N, 21.04. Found: C, 54.24; H, 6.81; N, 20.77.

Dihydrothiamin (X). (a) **By Reduction of Thiamin with Sodium Trimethoxyborohydride.**—A solution of 5.0 g. (0.015 mole) of thiamin chloride in 15 ml. of water at 0° was treated with 15 ml. (0.015 mole) of ice-cold *N* sodium hydroxide and 20 ml. of methanol. This solution was maintained at −12° during addition of 2.4 g. (0.019 mole) of sodium trimethoxyborohydride in small portions with mechanical stirring over a period of 0.5 hr. The cooling bath was removed and the mixture allowed to reach room temperature while still being stirred. Filtration afforded 2.1 g. (54%) of solid. This was recrystallized from 15 ml. of hot absolute alcohol to yield 1.6 g. (40%) of pure X, m.p. 151°.

Anal. Calcd. for $C_{12}H_{18}N_4OS$: C, 54.11; H, 6.82; N, 21.04. Found: C, 54.27; H, 6.91; N, 20.82.

(b) **By Reduction of Thiamin with Lithium Aluminum Hydride.**—Ten grams of thiamin chloride was added in small portions, in the course of 0.5 hr., with stirring to a suspension of 4 g. of lithium aluminum hydride in 120 ml. of anhydrous tetrahydrofuran. The reaction mixture was stirred at room temperature for 4 hr. and then treated with 8 ml. of water. The reaction mixture was filtered and the filtrate treated with CO_2 for five minutes and refiltered. The filtrate was evaporated to dryness under vacuum. The solid residue was recrystallized from 10 ml. of hot absolute alcohol to yield 0.8 g. (10%) of X, m.p. 145°. After two additional recrystallizations from absolute alcohol, the product melted sharply at 151°. The infrared spectrum was identical with that of a sample prepared by method a. The melting point of the mixed samples was 151°.

Anal. Calcd. for $C_{12}H_{18}N_4OS$: C, 54.11; H, 6.82; N, 21.04. Found: C, 54.25; H, 6.88; N, 20.56.

Conversion of Dihydrothiamin (X) to its Isomer IX.—Dihydrothiamin (X) (0.5 g.) was dissolved by heating in 5 ml. of water. The cooled solution was saturated with solid sodium carbonate and extracted several times with chloroform. The chloroform extract was dried over anhydrous potassium carbonate and evaporated to dryness. The residue was recrystallized from absolute alcohol–petroleum ether (b.p. 30–60°) to yield IX, m.p. 175°, yield 0.4 g.

Sulfite Cleavage of Dihydrothiamin (X).—Two grams of dihydrothiamin (X) were treated with sodium bisulfite at *p*H 5–5.5 as described in the above procedure for the sulfite cleavage of tetrahydrothiamin (II). The insoluble 2-methyl-4-aminopyrimidyl-5-methanesulfonic acid was recrystallized from hot water. The yield was 1.4 g. (90%).

Anal. Calcd. for $C_6H_9N_3O_3S$: C, 35.44; H, 4.46; N, 20.68; S, 15.76. Found: C, 35.25; H, 4.47; N, 20.76; S, 15.62.

Attempts to isolate the soluble sulfite cleavage product in pure form or as its picrate, picrolonate and hydrochloride were unsuccessful.

Potentiometric Titration.—A Coleman model 3A electrometer with glass electrode was used to follow *p*H changes. Approximately 100-mg. samples of tetrahydrothiamin dihydrobromide and of the dihydrothiamins IX and X were each dissolved in 50 ml. of carbon dioxide-free distilled water and titrated with standardized 0.1 *N* sodium hydroxide and 0.1 *N* hydrochloric acid, respectively, while nitrogen was bubbled into the solution to provide agitation and to exclude carbon dioxide. The basic pK_1 and pK_2 for II were 7.8 and 11.5, while those for IX and X were identical at 7.9 and 11.8.

Spectra.—Infrared spectra were taken by Miss Cecelia Vitiello of Schering Corp., Bloomfield, N. J., on a Perkin–Elmer double beam instrument using Nujol mulls. Ultraviolet spectra were taken on a Cary recording spectrophotometer using 2.0 mg. of solute/100 ml. of solution.

Cpd.	$\lambda_{max}^{H_2O}$, mμ	log ε	λ_{max}^{EtOH}, mμ	log ε
II	236	3.94	235	3.97
	270	3.75	278	3.69
IX	237	3.92	234	3.95
	280	3.81	278	3.70
X	237	3.93	243	3.93
	280	3.81	288	3.83

NEW YORK 58, N. Y.

[CONTRIBUTION FROM THE DANIEL SIEFF RESEARCH INSTITUTE, THE WEIZMANN INSTITUTE OF SCIENCE]

The Constituents of *Casimiroa edulis* Llave *et* Lex. III.[1] The Structure of Casimiroin[2]

BY ALEX MEISELS AND FRANZ SONDHEIMER

RECEIVED JULY 1, 1957

Casimiroin, a constituent of the seed and the bark of the tree *Casimiroa edulis* Llave *et* Lex., has been shown through degradation to be 1-methyl-4-methoxy-7,8-methylenedioxycarbostyril (IIIa).

In 1911, Power and Callan[3] described the results of a chemical investigation into the constituents of the seeds of the tree *Casimiroa edulis* Llave *et* Lex. (*Rutaceae*). Six substances (besides benzoic acid) were isolated, namely, casimiroedine (0.043%), casimiroin (0.0076%), casimirolid (0.060%), a "yellow phenolic substance" (0.004%), β-sitosterol β-D-glucoside ("ipuranol") (0.0078%) and β-sitos-

(1) Part II, see J. Iriarte, F. A. Kincl, G. Rosenkranz and F. Sondheimer, *J. Chem. Soc.*, 4170 (1956).

(2) Presented in part at the 21st Meeting of the Chemical Society of Israel, Jerusalem, April, 1957.

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

terol (0.024%). On the other hand, Aebi[4] has recently reported that he could obtain only casimiroedine from the seeds, the yield of this substance (0.45%) being over ten times that reported by Power and Callan. This worker therefore suggested that some of the substances isolated previously might have been artifacts which were not formed when the isolation was performed under mild conditions.

A systematic study of the constituents of the seeds has been carried out quite recently,[5] in the course of which all the six substances of Power and Callan[3] were isolated besides seven additional ones. The yield of casimiroedine was over twenty times that reported originally; the "yellow phenolic substance" (identified as 9-hydroxy-4-methoxyfurano-[3,2-g]benzopyran-7-one) was also obtained in increased yield, probably due to the improved isolation technique used involving chromatography, but all the other compounds were isolated in very much lower yields. The report of Aebi[4] regarding the yield of casimiroedine is therefore essentially correct and the fact that the other substances could not be found is probably because he worked with 2 kg. of seed whereas our work was performed with 100 kg. However, in view of the comparatively mild isolation conditions used by ourselves and in view of the structures so far known for the different substances, it is very unlikely that any of them are transformation products formed during the isolation procedure. The difference in yields between those obtained by Power and Callan and by ourselves must be due either to the different locations in which the seeds were collected or else the amount of the substances in the seed may have changed in the nearly 50-year interval between the two investigations.

Of the six substances obtained from the seed by Power and Callan[3] and by ourselves,[5] three (casimiroedine,[6] casimiroin and casimirolid) possess unknown structures. In this paper we describe the elucidation of the structure of casimiroin, a compound which is found not only in the seed[5] of *Casimiroa edulis* but also in the trunk and root bark.[1] The latter in fact is the best source of casimiroin, since it contains 0.22% of the substance, *viz.*, nearly two hundred times as much as the seed.

Casimiroin is a non-basic substance, m.p. 203°, $C_{12}H_{11}NO_4$, containing one methoxyl and one N-methyl group. The ultraviolet and infrared spectra have been described previously.[5] Power and Callan[3] had prepared two degradation products. The first, obtained by the action of boiling concentrated hydrochloric acid, was a phenolic substance, m.p. 323°, $C_{11}H_9NO_4$, which we have named casimiroinol[5] and shown to be derived from casimiroin by cleavage of the methoxyl group to hydroxyl since methylation regenerated the natural product. The second, casimiroitine,[7] m.p. 171°, was obtained by boiling casimiroin with alcoholic potassium hydroxide. In our previous publication[5] we considered that the presently known facts suggested casimiroin to be the methylamide of a methoxychromonecarboxylic acid. The infrared spectrum especially was in accord with this formulation. When we started our work on the complete structure of casimiroin, however, it soon became apparent that the suggested type of structure was untenable since the substance contained no active hydrogen. It therefore lacked the –CONHMe grouping and probably contained a tertiary amide function (–CONMe–).

Casimiroin on being boiled with sodium hydroxide in ethanol gave 11% of casimiroinol and 36% of a substance, m.p. 171°, which in view of its melting point, elemental composition and method of preparation must be the casimiroitine of Power and Callan.[3] Casimiroitine appeared to have the formula $C_{13}H_{13}NO_4$ (*viz.*, to contain one methylene group more than casimiroin), and both its infrared and ultraviolet spectra were very similar to those of the starting material. These facts suggested that ether interchange had taken place and that casimiroitine was merely the ethyl ether of the substance of which casimiroin was the methyl ether. That this was indeed the case was shown through the ethylation of casimiroinol with ethyl sulfate, whereby casimiroitine was smoothly obtained. Stronger treatment with potassium hydroxide did not lead to the desired breakdown in structure. Thus, boiling casimiroin with potassium hydroxide in ethylene glycol gave casimiroinol and a water-soluble product (probably the ethylene glycol ether of casimiroinol), whereas casimiroinol could be fused with potassium hydroxide at 210° without change. Strong mineral acids were equally without effect. It was already known that the action of boiling hydrochloric acid on casimiroin only caused the methyl ether grouping to be cleaved to give casimiroinol, and we have found that the latter can subsequently be boiled for several hours with hydriodic acid in acetic anhydride without change. The structure of casimiroin, with the exception of the methoxy group, is therefore remarkably stable under both acidic and basic conditions, and other reagents had to be found in order to obtain structural information.

The reduction of casimiroin with lithium aluminum hydride and isolation of the product in the usual way involving an acid treatment gave a yellow substance named for convenience Li 2, m.p. 109°, $C_{11}H_{11}NO_3$, which like casimiroin was not extracted from ether with mineral acids. Li 2 was ketonic (2,4-dinitrophenylhydrazone), had no hydroxyl function and no longer contained a methoxyl group. The loss of the elements of CO and the disappearance of the methoxyl and appearance of a keto group can best be rationalized by assuming that the lithium aluminum hydride has first converted the amide (–CONMe–) to the corresponding

(4) A. Aebi, *Helv. Chim. Acta*, **39**, 1495 (1956).

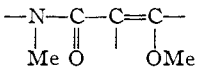
(5) F. A. Kincl, J. Romo, G. Rosenkranz and F. Sondheimer, *J. Chem. Soc.*, 4163 (1956).

(6) The empirical formula of casimiroedine, the structure of which is being investigated by Prof. C. Djerassi and co-workers and by Dr. A. Aebi, was originally given as $C_{17}H_{24}N_2O_6$ (footnote 3). The one analysis carried out by ourselves (footnote 5) supported this formula, but the correct formula has now been shown to be $C_{21}H_{27}N_3O_6$ (footnote 4; C. Djerassi, J. Herran, H. N. Khastgir, B. Riniker and J. Rome, *J. Org. Chem.*, **21**, 1510 (1956)).

(7) The formula $C_{23}H_{22}N_2O_7$ given to this substance by Power and Callan is of course incorrect since we have shown (footnote 5) that the formula $C_{24}H_{20}N_2O_8$ they proposed for casimiroin should be amended to $C_{13}H_{11}NO_4$.

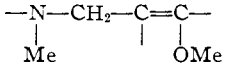
amine ($-CH_2NMe-$) in the usual way[8] and that the acid used during the isolation then caused the cleavage of an enol methyl ether to the corresponding ketone. The two-step nature of the process was confirmed when the lithium aluminum hydride reduction was carried out without a subsequent acid treatment. This procedure yielded the unstable Li 1, which differed from Li 2 but which was not investigated further.

It will be recalled that casimiroin contains a phenolic methyl ether grouping since concentrated hydrochloric acid gives the phenol casimiroinol. The fact that an enol methyl ether which can readily be cleaved to a ketone is obtained once the amide has been reduced to the amine shows that the carbonyl group of the amide function must be responsible for casimiroin to be hydrolyzed to a phenol rather than to a ketone. This suggested that casimiroin contains the grouping (a)



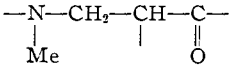
(a)

which on lithium aluminum hydride reduction first yields (b)



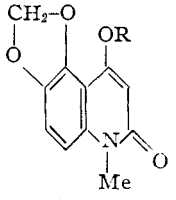
(b)

and thence on acid treatment (c)

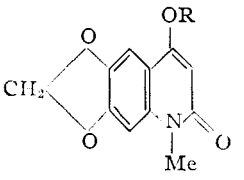


(c)

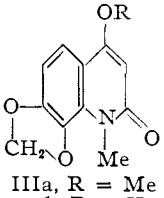
These considerations made a 1-methyl-4-methoxycarbostyril formulation likely for casimiroin. The surplus elements of CO_2 are best incorporated as a methylenedioxy grouping, and this leads to one of the three formulations Ia, IIa or IIIa (differing only



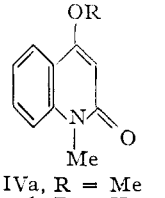
Ia, R = Me
b, R = H



IIa, R = Me
b, R = H



IIIa, R = Me
b, R = H
c, R = Et

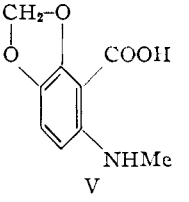


IVa, R = Me
b, R = H

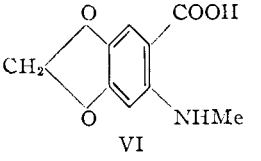
in the position of the methylenedioxy function) as the structure of casimiroin and Ib, IIb or IIIb as the structure for casimiroinol. The presence of the methylenedioxy grouping was confirmed by the fact that both casimiroin and Li 2 give a green color with alcoholic gallic acid and sulfuric acid.[9] This dioxolane grouping had not previously been suspected to be present in view of the very great stability of the ring system of casimiroin toward acids, including hydriodic acid. However, other examples of similar resistance of methylenedioxy functions to cleavage exist.[10]

The correctness of our conclusions up to this point could be confirmed by the following observations. Firstly, structure Ia, IIa or IIIa on lithium aluminum hydride reduction and acid treatment should give a ketone of type XVII with a vicinal methylene grouping, which was indeed formed since Li 2 gave a positive Zimmermann test and on condensation with anisaldehyde smoothly gave a red anisylidene derivative. Secondly, Li 2 (later shown to be XVII) is presumably not extractable from ether with acids since it is a vinylogous amide. As expected, a second lithium aluminum hydride reduction of Li 2 gave the dihydro derivative Li 3 (later shown to be XVIII) in which the ketone has been reduced to the alcohol and which now was basic. Thirdly, the known 1-methyl-4-hydroxycarbostyril (IVb)[11] was prepared from N-methylanthranilic acid with acetic anhydride[11b] and hence 1-methyl-4-methoxycarbostyril (IVa)[11a,c] by methylation. The infrared spectra of both IVa and IVb showed a striking similarity to those of casimiroin and casimiroinol, respectively. Casimiroin therefore doubtless is a methylenedioxy-1-methyl-4-methoxycarbostyril, and it was now necessary to distinguish between the structures Ia, IIa and IIIa, *viz.*, to locate the points of attachment of the methylenedioxy grouping.

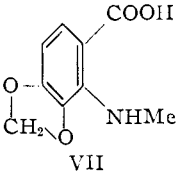
Toward this end casimiroinol was treated with alkaline hydrogen peroxide, whereby for the first time ring cleavage occurred. An amino-acid, m.p. 181°, $C_9H_9NO_4$, was obtained which still contained the N-methyl grouping. It could be converted to a methyl ester and thence to a methyl ester N-acetyl derivative, and it must be one of the three methylenedioxy-N-methylanthranilic acids V, VI or VII. As expected, lithium aluminum hydride



V



VI



VII

reduction gave the corresponding benzyl alcohol, whereas heating with acetic anhydride (*cf.* footnote 11b) and subsequent methylation regenerated casi-

(8) *Cf.* W. G. Brown, "Organic Reactions," Vol. VI, John Wiley and Sons, Inc., New York, N. Y., 1951, Chapter 10, pp. 479–480; N. G. Gaylord, "Reduction with Complex Metal Hydrides," Interscience Publishers, Inc., New York, N. Y., 1956, pp. 544–592.

(9) *Cf.* M. A. Labat, *Bull. soc. chim., France*, [4] **5**, 745 (1909).

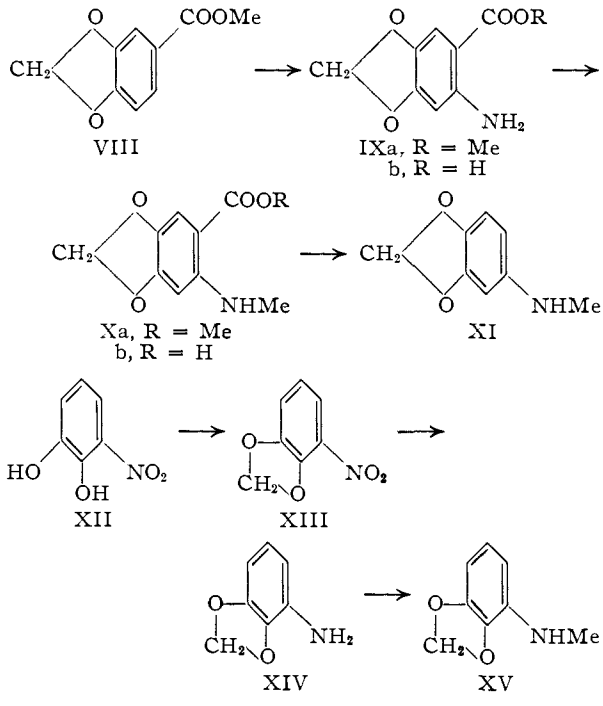
(10) *Cf.* W. Borsche and J. Niemann, *Ann.*, **494**, 126 (1932).

(11) (a) P. Friedländer and F. Müller, *Ber.*, **20**, 2009 (1887); (b) Farbwerk vorm. Meister Lucius and Bruning, German Patent 287,803 [P. Friedländer, *Fortschritte der Teerfarbenfabrikation*, **12**, 153 (1914)]; (c) F. Arndt, L. Ergener and O. Kutlu, *Ber.*, **86**, 951 (1953). These latter authors showed substance IVa and its methyl ether IVb to exist in the 4 substituted 1-methylcarbostyril rather than in the 2-substituted-1-methylkynurine form.

miroin; the interrelationship between the amino-acid and casimiroin is thereby conclusively established. The stability of the methylenedioxy grouping is again shown by the fact that the amino-acid was unaffected by being heated with Raney nickel–aluminum alloy and aqueous sodium hydroxide, conditions which smoothly reduce other methylenedioxybenzene derivatives to the corresponding phenols [*e.g.*, piperonylic acid (VII without the methylamino group) to *m*-hydroxybenzoic acid].[12]

The methylamino-acid V, VI or VII on being heated with copper powder readily underwent decarboxylation. The product should be 3,4-methylenedioxy-N-methylaniline (XI) if the precursor had structure V or VI or 2,3-methylenedioxy-N-methylaniline (XV) if the precursor was VII. Neither XI nor XV was known and consequently both were synthesized.

For the preparation of 3,4-methylenedioxy-N-methylaniline (XI), methyl piperonylate (VIII) was nitrated and then reduced to methyl 6-aminopiperonylate (IXa), as described previously.[13] Saponification to 6-aminopiperonylic acid (IXb) and subsequent methylation with 1 equivalent of methyl sulfate yielded 6-methylaminopiperonylic acid (Xb), which on being heated with copper powder underwent decarboxylation and produced 3,4-methylenedioxy-N-methylaniline (XI). The infrared spectrum of the last mentioned substance

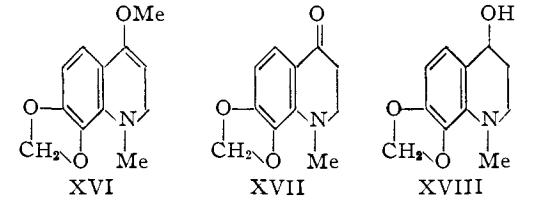


differed from that of the final degradation product of casimiroin, and the methylamino-acid obtained by the hydrogen peroxide oxidation of casimiroinol therefore cannot have the structure V or VI. The latter formulation is moreover excluded since the methylamino-acid from casimiroinol is different from the synthetic acid Xb.

(12) E. Schwenk and D. Papa, *J. Org. Chem.*, **10**, 232 (1945).

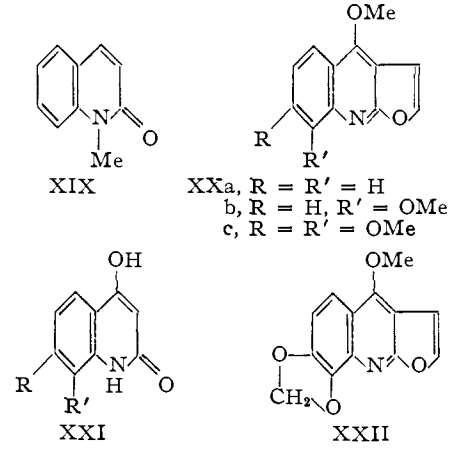
(13) E. Oertly and A. Pictet, *Ber.*, **43**, 1336 (1910); H. Kondo, Japanese Patent, 7220 ('51) (*C.A.*, **48**, 726 (1954)).

For the synthesis of 2,3-methylenedioxy-N-methylaniline (XV), we employed 3-nitrocatechol (XII) (obtained together with 4-nitrocatechol by the nitration of catechol)[14] as starting material. Heating XII with methylene iodide and sodium ethoxide in ethanol yielded 2,3-methylenedioxynitrobenzene (XIII). This substance on hydrogenation in acetic acid over platinum oxide gave 2,3-methylenedioxyaniline (XIV), which on methylation with methyl sulfate in water suspension was transformed to 2,3-methylenedioxy-N-methylaniline (XV). The latter was identical in every respect with the final degradation product of casimiroin. The methylamino-acid obtained from casimiroinol with hydrogen peroxide is therefore 3,4-methylenedioxy-N-methylanthranilic acid (VII), casimiroinol is 1-methyl-4-hydroxy-7,8-methylenedioxycarbostyril (IIIb) and casimiroin itself is 1-methyl-4-methoxy-7,8-methylenedioxycarbostyril (IIIa). Furthermore casimiroitine is 1-methyl-4-ethoxy-7,8-methylenedioxycarbostyril (IIIc), Li 1 is 1-methyl-4-methoxy-7,8-methylenedioxy-1,2-dihydroquinoline (XVI), Li 2 is 1-methyl-4-keto-7,8-methylenedioxy-1,2,3,4-tetrahydroquinoline



(XVII)[15] and Li 3 is 1-methyl-4-hydroxy-7,8-methylenedioxy-1,2,3,4-tetrahydroquinoline (XVIII).

In the structure of casimiroin (IIIa) one can recognize a close relationship to a number of other alkaloids also isolated from plants belonging to the *Rutaceae* family.[16] For instance 1-methylcarbostyril (XIX) has been found in Angostura bark[17]



and the quinoline nucleus oxygenated at the C-4 as well as at the C-2 position is known to occur in

(14) P. Weselky and R. Benedikt, *Monatsh.*, **3**, 386 (1882); F. L. Gilbert, F. C. Laxton and E. B. R. Prideaux, *J. Chem. Soc.*, 2295 (1927).

(15) 1-Methyl-4-keto-1,2,3,4-tetrahydroquinoline (XVII without the methylenedioxy group) has been described for the first time recently by J. A. C. Allison, J. T. Braunholtz and F. G. Mann (*J. Chem. Soc.*, 403 (1954)).

(16) *Cf.* J. R. Price, *Fortschr. Chem. org. Naturstoffe*, **13**, 302 (1956).

(17) E. Späth and J. Pikl, *Monatsh.*, **55**, 352 (1930).

flindersine[18] and in all the furoquinoline alkaloids. Three of the latter, namely, dictamnine (XXa), γ-fagarine (XXb) and skimmianine (XXc) have previously been isolated by us from the bark of *Casimiroa edulis*.[1] In particular the relationship between casimiroin and skimmianine is noteworthy, especially since it is known that in the laboratory furoquinolines of type XX can be degraded to 4-hydroxycarbostyrils (type XXI)[19] through successive potassium permanganate oxidation and acid treatment.[20] It is also of interest that the furoquinoline alkaloid kokusagine (XXII)[21] appears to contain the same 4-methoxy-7,8-methylenedioxy substituents as does casimiroin.

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Experimental[22]

Casimiroitine (1-Methyl-4-ethoxy-7,8-methylenedioxycarbostyril) (IIIc). (a) From Casimiroin.—A solution containing 0.5 g. of casimiroin[5] and 2.5 g. of sodium hydroxide in 25 cc. of ethanol was boiled under reflux for 14 hr. The crystalline product (0.44 g., m.p. 150–165°), obtained by removal of the solvent under reduced pressure followed by addition of water, was chromatographed on 15 g. of alumina. Elution with benzene and crystallization from acetone–hexane gave 0.19 g. (36%) of casimiroitine, m.p. 170–171°; λ_{max} 227, 252, 260 and 303 mμ (log ε 4.40, 4.23, 4.21 and 3.70, respectively); ν_{max} 1642 and 1592 cm.$^{-1}$; reported[3] m.p. 171°, C, 63.1; H, 5.4; N, 6.6.

Anal. Calcd. for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.30; N, 5.67; O, 25.89. Found: C, 63.45; H, 5.41; N, 5.66; O, 25.60.

Further elution of the column with benzene and with benzene–ether yielded 0.26 g. (52%) of unchanged casimiroin. The alkaline filtrate, obtained after removal of the 0.44 g. of solid, was acidified. The resulting precipitate (0.05 g., 11%) after crystallization from methanol showed m.p. 320–322° and was shown to be casimiroinol (IIIb), since it was identical (mixture m.p., infrared comparison) with an authentic sample (m.p. 321–323°).[5]

(b) By Ethylation of Casimiroinol.—Casimiroinol[5] (50 mg.) was dissolved in 5 cc. of 20% aqueous sodium hydroxide, 2 cc. of ethyl sulfate was added and the mixture was shaken vigorously at 60° for 24 hr. The resulting precipitate, after collection and crystallization from acetone–hexane, yielded 31 mg. of casimiroitine, m.p. 170–171°, identified with the material from casimiroin by mixture m.p. determination and infrared comparison.

Lithium Aluminum Hydride Reduction of Casimiroin to 1-Methyl-4-methoxy-7,8-methylenedioxy-1,2-dihydroquinoline (XVI) and 1-Methyl-4-keto-7,8-methylenedioxy-1,2,3,4-tetrahydroquinoline (XVII).—A solution of 2 g. of casimiroin in 200 cc. of dry tetrahydrofuran was added dropwise under nitrogen to a stirred solution of 4 g. of lithium aluminum hydride in 100 cc. of tetrahydrofuran during 15 minutes. The mixture was boiled under reflux for 7 hr., cooled and poured onto excess ice-cold dilute sulfuric acid. The product was extracted with ether and the solid residue remaining after removal of the solvent was crystallized from acetone–hexane. This procedure yielded 1.41 g. of 1-methyl-4-keto-7,8-methylenedioxy-1,2,3,4-tetrahydroquinoline (XVII) as yellow prisms, m.p. 108–109°; λ_{max} 250 and 301 mμ (log ε 4.23 and 3.78, respectively); ν_{max} 1669, 1621 and 1577 cm.$^{-1}$. The substance gave a green color with sulfuric acid and alcoholic gallic acid.[9] It could not be extracted out of ether by means of dilute hydrochloric acid or sulfuric acid.

Anal. Calcd. for $C_{11}H_{11}NO_3$: C, 64.38; H, 5.40; N, 6.83; 1 (N)Me, 7.31. Found: C, 64.58; H, 5.40; N, 6.83; (N)Me, 6.82; act. H, 0.0; OMe, 0.0.

The 2,4-dinitrophenylhydrazone crystallized from methylene chloride–methanol as brown-red needles which decomposed on heating.

Anal. Calcd. for $C_{17}H_{15}N_5O_6$: C, 52.99; H, 3.92; N, 18.18. Found: C, 53.01; H, 3.69; N, 18.57.

In another reduction experiment carried out as above, the reaction mixture instead of being poured into acid was carefully diluted with 100 cc. of ethyl acetate. Saturated sodium sulfate solution was then added until the precipitate began to adhere to the sides of the flask. The mixture was diluted with ether, dried over sodium sulfate, filtered and the filtrate was evaporated. The crystalline XVI which remained showed an infrared spectrum (ν_{max} 1664, 1626 and 1600 cm.$^{-1}$) completely different from that of XVII.

1-Methyl-3-anisylidene-4-keto-7,8-methylenedioxy-1,2,3,4-tetrahydroquinoline.—A stream of dry hydrogen chloride was passed for 2 hr. through a solution of 300 mg. of 1-methyl-4-keto-7,8-methylenedioxy-1,2,3,4-tetrahydroquinoline (XVII) and 0.3 cc. of anisaldehyde in 3 cc. of glacial acetic acid, kept at 0°. The deep red solution was allowed to stand at 0° for 2 days and was then diluted with water. The resulting precipitate was collected, washed well with water, dried and crystallized from methylene chloride–methanol. The anisylidene derivative thus obtained weighed 295 mg. and formed red needles, m.p. 135–138°; λ_{max} 232 and 332 mμ (log ε 4.49 and 4.47, respectively).

Anal. Calcd. for $C_{19}H_{17}NO_4$: C, 70.57; H, 5.30; N, 4.33; (N)Me and (O)Me, 9.28. Found: C, 70.66; H, 5.16; N, 4.26; (N)Me and (O)Me, 9.42.

1-Methyl-4-hydroxy-7,8-methylenedioxy-1,2,3,4-tetrahydroquinoline (XVIII).—A solution of 400 mg. of 1-methyl-4-keto-7,8-methylenedioxy-1,2,3,4-tetrahydroquinoline (XVII) in 80 cc. of dry tetrahydrofuran was added slowly under nitrogen to 2 g. of lithium aluminum hydride in 40 cc. of tetrahydrofuran, and the mixture was boiled under reflux for 6 hr. It then was cooled, poured on ice and 50 cc. of a saturated ammonium chloride solution was added. The mixture was extracted with ether, the ether layer was shaken with 10% sulfuric acid and the acid extract was made alkaline with sodium hydroxide and again extracted with ether. This last ether extract was washed with water, dried and the liquid residue (398 mg.) was chromatographed on 10 g. of alumina. Pentane–benzene (1:1) eluted the substituted tetrahydroquinoline XVIII (320 mg.) as a liquid, b.p. 100–105° (bath temp.) (0.08 mm.); λ_{max} 230 and 258 mμ (log ε 3.45 and 2.89, respectively); ν_{max} 1637 and 1590 cm.$^{-1}$.

Anal. Calcd. for $C_{11}H_{13}NO_3$: C, 63.75; H, 6.32; 1 act. H, 0.48. Found: C, 63.86; H, 6.40; act. H, 0.42.

Hydrogen Peroxide Oxidation of Casimiroinol to 3,4-Methylenedioxy-N-methylanthranilic Acid (VII).—Hydrogen peroxide solution (100 cc., 30%) was added to a solution of 1 g. of casimiroinol in 50 cc. of 10% aqueous sodium hydroxide. After being allowed to stand for 24 hr. at 30°, the reaction mixture was diluted with excess dilute hydrochloric acid, a small amount of precipitate was removed by filtration, the filtrate was saturated with sodium chloride and thoroughly extracted with ether. The ether solution was washed with sodium bicarbonate solution and the latter was then acidified and thoroughly extracted with ether. This last ether extract was dried, evaporated and the semi-solid residue (0.59 g.) was sublimed in vacuum. The main fraction which sublimed at 115–130° (bath temp.) (0.08 mm.), was crystallized from acetone–hexane and yielded 0.37 g. of the methylamino-acid VII as needles, m.p. 180–181° dec.; λ_{max} 238, 276 and 339 mμ (log ε 4.37, 3.71 and 3.40, respectively); ν_{max} 1669 and 1597 cm.$^{-1}$.

(18) R. F. C. Brown, J. J. Hobbs, G. K. Hughes and E. Ritchie, *Australian J. Chem.*, **7**, 348 (1954).

(19) For the formulation of this type of substance as a 4-hydroxy-2-quinolone rather than a 2,4-dihydroxyquinoline *cf.* footnote 11c.

(20) Y. Asahina, T. Ohta and M. Inubuse, *Ber.*, **63**, 2045 (1930); Y. Asahina and M. Inubuse, *ibid.*, **63**, 2052 (1930).

(21) M. Terasaka, T. Ohta and K. Narahaski, *Pharm. Bull. (Japan)*, **2**, 159 (1954) (*C.A.*, **50**, 1054 (1956)).

(22) Melting points are uncorrected. Ultraviolet spectra were measured in 95% ethanol solution on a Unicam model S.P. 500 spectrophotometer and infrared spectra in chloroform solution (unless otherwise mentioned) on a Baird double-beam recording spectrophotometer [for brevity only bands in the carbonyl region (1800–1500 cm.$^{-1}$) are recorded]. All chromatograms were made using acid-washed reagent grade aluminum oxide (Merck). Analyses were carried out in our microanalytical department under the direction of Mr. Erich Meier.

Anal. Calcd. for $C_9H_9NO_4$: C, 55.38; H, 4.65; N, 7.18; 1 (N)Me, 7.70; 2 act. H, 1.03. Found: C, 55.34; H, 4.52; N, 7.22; (N)Me, 8.13; act. H, 1.00.

The methyl ester was prepared by treating the acid VII in ether solution with diazomethane at 5° overnight. It crystallized from acetone–hexane as prisms, m.p. 91°; ν_{max} 1683, 1629 and 1597 cm.$^{-1}$.

Anal. Calcd. for $C_{10}H_{11}NO_4$: C, 57.41; H, 5.30; N, 6.70. Found: C, 57.54; H, 5.38; N, 6.92.

The methyl ester N-acetyl derivative was prepared by treating the methyl ester (50 mg.) in pyridine (2 cc.) with acetyl chloride (0.5 cc.) overnight. It was isolated with ether and after crystallization from this solvent formed prisms, m.p. 91–92°, ν_{max} 1718, 1661 and 1629 cm.$^{-1}$.

Anal. Calcd. for $C_{12}H_{13}NO_5$: C, 57.37; H, 5.22. Found: C, 57.39; H, 5.41; act. H, 0.0.

2-Methylamino-3,4-methylenedioxybenzyl Alcohol.—The methylamino-acid VII (200 mg.) in 20 cc. of tetrahydrofuran was boiled for 16 hr. with 1 g. of lithium aluminum hydride in 30 cc. of tetrahydrofuran under nitrogen. The procedure used for the isolation of the basic product was exactly that described above for the conversion of XVII to XVIII. Crystallization from ether–hexane gave 175 mg. of the benzyl alcohol as needles, m.p. 94–95°, ν_{max} 1642 cm.$^{-1}$.

Anal. Calcd. for $C_9H_{11}NO_3$: C, 59.66; H, 6.11; N, 7.73. Found: C, 59.71; H, 6.02; N, 7.80.

Reconversion of 3,4-Methylenedioxy-N-methylanthranilic Acid (VII) to Casimiroin.—The methylamino-acid VII (100 mg.) and acetic anhydride (0.3 cc.) were heated at 75° for 30 minutes with the exclusion of moisture. The mixture was diluted with ice and water and extracted with ethyl acetate. The organic extract was washed with sodium bicarbonate solution and water and then was dried and evaporated. The resulting crude casimiroinol (15 mg.) could not be crystallized, and it was therefore dissolved in 20 cc. of methanol and allowed to stand with excess ethereal diazomethane overnight at 5°. The solvents then were removed, the residue was dissolved in ether, washed with sodium hydroxide solution and water, dried and evaporated. The residue (16 mg.) was chromatographed on 400 mg. of alumina; the fractions eluted with ether on crystallization from acetone–hexane gave 10 mg. of casimiroin as needles, m.p. 200–201°. The substance was identified with authentic material through mixture m.p. determination and infrared comparison.

2,3-Methylenedioxy-N-methylaniline (XV) (by Degradation).—3,4-Methylenedioxy-N-methylanthranilic acid (VII) (100 mg.), obtained by the oxidation of casimiroinol with hydrogen peroxide, was heated with 10 mg. of copper powder at 200–205° for 10 minutes. After being allowed to cool, the mixture was extracted with ether and the extract was filtered and evaporated. Two distillations of the residue in high vacuum gave 59 mg. of 2,3-methylenedioxy-N-methylaniline as a colorless liquid, b.p. 115–120° (bath temp.) (0.08 mm.), $n^{23}D$ 1.5765, $\nu_{max}^{CCl_4}$ 1650 cm.$^{-1}$. A bluish-green color was obtained with alcoholic ferric chloride.

Anal. Calcd. for $C_8H_9NO_2$: C, 63.56; H, 6.00; N, 9.27. Found: C, 63.39; H, 6.11; N, 9.11.

6-Methylaminopiperonylic Acid (Xb).—Methyl 6-aminopiperonylate (IXa)[13] (2 g., m.p. 107–108°) was saponified to the acid IXb through being boiled for 15 minutes with a solution of 0.46 g. of sodium hydroxide in 16 cc. of water. The clear solution was cooled, methyl sulfate (1.12 cc., 1.15 equivalents) was added and the mixture was shaken for 2 hr. The resulting precipitate was collected, washed well with water and cold methanol and dried. Crystallization from methanol gave 6-methylaminopiperonylic acid (1.35 g.) as needles, m.p. 183–184° dec.; λ_{max} 238, 267 and 367 mμ (log ε 4.20, 3.80 and 3.81, respectively); ν_{max}^{mull} 1667 and 1597 cm.$^{-1}$. There was a large depression in m.p. on admixture with the methylamino-acid VII derived from casimiroinol and the infrared spectra were different.

Anal. Calcd. for $C_9H_9NO_4$: C, 55.38; H, 4.65; N, 7.18; 1 (N)Me, 7.70. Found: C, 55.11; H, 4.46; N, 6.96; (N)Me, 8.02.

The methyl ester was prepared by treating a solution of the acid Xb in methanol and ether with ethereal diazomethane at 5° overnight. It crystallized from acetone–hexane as prisms, m.p. 122°, ν_{max} 1678 and 1615 cm.$^{-1}$. The substance was shown to differ from the methyl ester of the acid VII derived from casimiroinol since there was a depression in m.p. on admixture and since the infrared spectra were different.

Anal. Calcd. for $C_{10}H_{11}NO_4$: C, 57.41; H, 5.30; N, 6.70. Found: C, 57.51; H, 4.93; N, 6.53.

3,4-Methylenedioxy-N-methylaniline (XI).—An intimate mixture of 6-methylaminopiperonylic acid (Xb) (100 mg.) and copper powder (10 mg.) was heated at 210–220° for 15 minutes. The cooled melt was extracted with ether, the extract was filtered and evaporated. Two distillations of the residue in vacuum afforded 61 mg. of 3,4-methylenedioxy-N-methylaniline as a colorless liquid, b.p. 100–105° (bath temp.) (0.07 mm.), $n^{23}D$ 1.5791, $\nu_{max}^{CCl_4}$ 1634 cm.$^{-1}$. A red color was obtained with alcoholic ferric chloride. The infrared spectrum was different from that of the methylenedioxy-N-methylaniline derived from casimiroin.

Anal. Calcd. for $C_8H_9NO_2$: C, 63.56; H, 6.00; N, 9.27. Found: C, 63.64; H, 6.00; N, 9.36.

2,3-Methylenedioxynitrobenzene (XIII).—3-Nitrocatechol (XII)[14] (6 g., m.p. 86°) and methylene iodide (15 g.) were added to a solution of 1.8 g. of sodium in 50 cc. of absolute alcohol, under nitrogen. The mixture was then heated under reflux for 20 hr. in nitrogen, the alcohol was removed under reduced pressure, water was added to the residue and the product was extracted with ether. The ether solution was washed successively with sodium hydroxide solution, sodium thiosulfate solution and water and was then dried and evaporated. Distillation of the excess methylene iodide under reduced pressure, and crystallization of the residue from methanol gave 0.94 g. of 2,3-methylenedioxynitrobenzene as yellow needles, m.p. 118°.

Anal. Calcd. for $C_7H_5NO_4$: C, 50.31; H, 3.02; N, 8.38. Found: C, 50.09; H, 3.13; N, 8.31.

2,3-Methylenedioxyaniline (XIV).—A solution of 420 mg. of 2,3-methylenedioxynitrobenzene (XIII) in 10 cc. of glacial acetic acid was shaken with 60 mg. of 10% palladium–charcoal in hydrogen for 16 hr. at 23° and *ca.* 2 atmospheres pressure. The catalyst was removed, the filtrate was made alkaline by the addition of 100 cc. of a 10% sodium hydroxide solution and extracted with ether. The ether solution was washed with water, dried and evaporated. Distillation of the residue under reduced pressure gave 2,3-methylenedioxyaniline (240 mg.) as a colorless liquid, b.p. 100–105° (bath temp.) (0.09 mm.).

Anal. Calcd. for $C_7H_7NO_2$: C, 61.31; H, 5.15. Found: C, 61.59; H, 5.31.

2,3-Methylenedioxy-N-methylaniline (XV) (by Synthesis).—2,3-Methylenedioxyaniline (XIV) (220 mg.) was shaken with 20 cc. of water and 0.15 cc. of methyl sulfate for 16 hr. at room temperature. The mixture was made alkaline by the addition of dilute sodium hydroxide and extracted with ether. The organic extract was washed with water, dried and evaporated. The residue was chromatographed on 6 g. of alumina and the liquid eluted with hexane was distilled under reduced pressure. This procedure yielded 107 mg. of 2,3-methylenedioxy-N-methylaniline as a colorless liquid, b.p. 115–120° (bath temp.) (0.08 mm.), $n^{23}D$ 1.5765, $\nu_{max}^{CCl_4}$ 1650 cm.$^{-1}$. A bluish-green color was obtained with alcoholic ferric chloride. The substance was identical with the methylenedioxy-N-methylaniline derived from casimiroin since the infrared spectra were superimposable in every detail.

Anal. Calcd. for $C_8H_9NO_2$: C, 63.56; H, 6.00; N, 9.27. Found: C, 63.54; H, 5.91; N, 9.48.

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