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Power, Frederick B. 1853-1927.
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Publication/Creation

London : Wellcome Chemical Research Laboratories, 1911.

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THE CONSTITUENTS
OF THE SEEDS OF
CASIMIROA EDULIS

BY
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AND
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(From the Transactions of the Chemical Society, Vol. 99, 1911)



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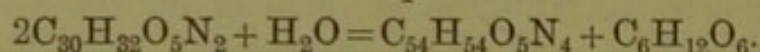
CCXXVI.—*The Constituents of the Seeds of Casimiroa edulis.*

By FREDERICK BELDING POWER and THOMAS CALLAN.

THE *Casimiroa edulis*, La Llave and Lejarza (Nat. Ord. *Rutaceae*), is a tree which is widely distributed throughout Mexico and Central America. It is known in Mexico by the vernacular names of "Chochitzapotl" and "Iztactzapotl," and in Guatemala as "Mato sano." Under the title of "Zapote blanco," the Pharmacopœia of Mexico recognises the fruit and the seed of the *Casimiroa*, indicating the former to be used as an anthelmintic and the latter as a vulnerary, for which purpose the kernels of the seed are roasted and powdered. The fruit, as the specific name of the plant denotes, is edible, and has an agreeable flavour, although it is stated to induce sleep, whereas it has been recorded that the kernels of the seed are deleterious or even fatal in their effects (Hernandez, *Rerum medicarum Novae Hispaniae thesaurus*, etc., *Romae*, 1651, *Lib. III.*, p. 89).

The first chemical investigation of the seed of the *Casimiroa* was by José Sanchez, who, in a thesis published in Mexico in 1893, indicated the presence of a crystalline substance giving the general reactions of an alkaloid, together with two resins, an essential oil, fatty matter, gum, glucose, and starch. The alkaloidal substance was considered to be the probable source of the reputed physiological action of the seed. In 1898 a Commission was appointed in Mexico for the botanical and chemical study of the *Casimiroa*, and for the purpose of determining its therapeutic value. As a result of this inquiry, it was stated by Altamirano that the seed contains a glucoside, which, however, could be obtained only as a pale yellow, amorphous mass. This product, which he regarded as the active principle, possessed a taste which was at first sweet, but afterwards bitter and persistent. It was soluble in water and in alcohol, and its solution yielded precipitates with the usual alkaloid reagents. The results of a subsequent study by Bocquillon, in France, were incorporated in a thesis entitled, "Étude botanique et pharmacologique des Xanthoxyléas-Casimiroa," 1901, pp. 104, and more recently the subject has been investigated by Bickern (*Arch. Pharm.*, 1903, **241**, 166). A series of pharmacological investigations relating to the *Casimiroa* has furthermore been recorded by Robin and Coyon, by Chevalier, and by Vincent in *Bull. gén. de thérapeutique*, 1909, **158**, 16, 96, 193, 241, the last-mentioned author having also given a complete historical survey of the subject.

The chemical examination of *Casimiroa* seeds by Bickern (*loc. cit.*) was apparently conducted with a very small quantity of material, the amount not being directly stated; but from the results obtained he was led to conclude that they contain a "gluco-alkaloid," melting at 106° , to which he assigned the name "casimirin," and the formula $C_{30}H_{32}O_5N_2$. This formula, however, was based on but one analysis, and its theoretical percentage figures were incorrectly recorded. Nevertheless, the substance was stated to undergo hydrolysis by heating with 30 per cent. hydrochloric acid, with the formation of an alkaloid, $C_{54}H_{54}O_5N_4$, and dextrose, in accordance with the equation:



In this case also a considerable discrepancy in the figures appears to exist, inasmuch as an analysis of the residual alkaloid indicated it to contain 73.2 per cent. of carbon, whereas the formula $C_{54}H_{54}O_5N_4$ requires C=77.3 per cent. It would, moreover, be impossible for a substance of the composition $C_{30}H_{32}O_5N_2$ to yield dextrose on hydrolysis, inasmuch as it contains but five oxygen atoms.

Another compound described by Bickern (*loc. cit.*) was obtained by mixing the powdered seed with lime, and extracting with ether, when, on evaporating the solvent, a colourless substance was precipitated. The latter, when crystallised from chloroform, separated in colourless needles, melting at 207° , and to it the name "casimirool" and the formula $C_{27}H_{48}O_2$ were assigned. As this substance yielded colour reactions similar to those of the phytosterols, it was assumed to be related to that class of compounds.

It was furthermore noted by Bickern that the above-mentioned substances, as well as various ethereal and alcoholic extracts of the seed, were tested on animals by Professor Cloëtta, of Zurich, but were found to be devoid of any hypnotic action. On the other hand, Chevalier (*loc. cit.*, p. 96) was led to conclude from the results of his experiments on animals that the therapeutic value of *Casimiroa* seed is due to the essential oil and resin they contain (compare also *Lancet*, August 21st, 1909, p. 561).

From the preceding brief review of the literature it will be observed that the statements respecting the constituents and physiological action of *Casimiroa* seeds are very divergent, and, to a large extent, inconclusive. In view of the interest pertaining to the subject it was deemed desirable to submit these seed to a more complete examination, and the results are embodied in the present communication.

EXPERIMENTAL.

Inasmuch as the entire fruit of *Casimiroa edulis* could not conveniently be transported to this country, and the seed are considered to be the most active portion, a quantity of the latter was specially collected in Mexico for the purpose of the present investigation. The seed, which in size and shape somewhat resemble an almond, are provided with a grey, fibrous shell, enclosing a kernel, which is covered with a thin, brown membrane. The shells were found to represent about one-fifth the weight of the entire fresh seed, and they were separately examined.

I.—*Examination of the Kernels.**Separation of an Enzyme.*

A quantity (810 grams) of the kernels, representing 1 kilogram of the entire seed, was crushed, and macerated with water at the ordinary temperature for two days, the liquid being then expressed and filtered. To the filtered liquid, in which the presence of starch was indicated, about twice its volume of alcohol was added, when an abundant flocculent precipitate was produced. After about twenty-four hours the precipitate was collected, washed with a little alcohol, and dried, first on a porous plate, and finally in a vacuum over sulphuric acid. It could then be reduced to a light brown powder, and amounted to 9 grams, or 0.9 per cent. of the weight of entire seed employed.

The above-described product, when dissolved in water, yielded a solution which frothed strongly on agitation, and gave an abundant, white, curdy precipitate, both with hydrochloric acid and on boiling. It gave the biuret reaction, and also slowly hydrolysed amygdalin, thus proving the presence of an enzyme.

Preliminary Test for an Alkaloid.—A small portion (about 6 grams) of the dried kernels of the seed was treated with Prollius' fluid, and the resulting liquid tested in the usual manner for an alkaloid. The results obtained indicated the presence of a considerable proportion of such a substance.

For the purpose of a complete examination of the seed, the shells were first separated from the kernels, and a quantity of the latter, which, after being ground, amounted to 37.05 kilograms, was extracted by continuous percolation with hot alcohol until exhausted. In view of the asserted presence of a glucoside, or of a "glucoalkaloid," in the seed, and in order to preclude the hydro-

lysis of such a substance, all the preliminary operations were conducted as quickly as possible. After the removal of the greater portion of the alcohol, the amount of thin extract obtained was 11.45 kilograms.

Distillation of the Extract with Steam. Separation of an Essential Oil.

The entire amount of extract was subjected to distillation in a current of steam, and the distillate, which contained some oily drops in suspension, extracted with ether. After the removal of the solvent, a quantity (8 grams) of an oily liquid was obtained, which, when distilled under diminished pressure, passed over between 80° and $160^{\circ}/25$ mm., but for the most part below $130^{\circ}/25$ mm. This essential oil, when freshly distilled, had a pale yellow colour, but gradually darkened on keeping, even in absence of air. It possessed an agreeable, aromatic odour, and the following constants: $d=0.9574$ at 20° ; $\alpha_D -2^{\circ}25'$ in a 25 mm. tube. It gave no coloration with ferric chloride, and did not respond to the test for furfuraldehyde.

Non-volatile Constituents of the Extract.

After the above-described operation, there remained in the distillation vessel a dark-coloured aqueous liquid (A), together with a quantity of a soft, oily resin (B). These products were separated, and the resin thoroughly washed with hot water, the washings being added to the main portion of the aqueous liquid.

Examination of the Aqueous Liquid (A).

The aqueous liquid was repeatedly extracted with large quantities of ether, and the ethereal liquid, after concentrating to a convenient bulk, was shaken successively with aqueous ammonium carbonate, sodium carbonate, and sodium hydroxide.

Isolation of a New Alkaloid, Casimiroine, $C_{24}H_{20}O_8N_2$.

On shaking the ethereal extract of the aqueous liquid with a concentrated solution of ammonium carbonate, a crystalline substance slowly separated, and remained suspended in the alkaline liquid. This substance was collected, and amounted to 2.8 grams. It crystallised very readily from alcohol or ethyl acetate, separating in rosettes of colourless needles, and melted at $196-197^{\circ}$. Although very sparingly soluble in water, its aqueous solution, when acidified, gave copious precipitates with the usual alkaloidal reagents, such

as solutions of iodine in potassium iodide, potassium-mercuric iodide, and picric acid:

0.1170 gave 0.2670 CO_2 and 0.0514 H_2O . $\text{C}=62.2$; $\text{H}=4.9$.

0.1322 ,, 7.2 c.c. N_2 at 16° and 768 mm. $\text{N}=6.4$.

$\text{C}_{24}\text{H}_{20}\text{O}_8\text{N}_2$ requires $\text{C}=62.1$; $\text{H}=4.3$; $\text{N}=6.0$ per cent.

These results, together with a subsequent analysis of its aurichloride, indicate the above-described alkaloid to possess the formula $\text{C}_{24}\text{H}_{20}\text{O}_8\text{N}_2$, and, being a new compound, it is proposed to designate it *casimiroine*.

Casimiroine contains two methoxyl groups, as the following determination by Perkins's modification of the Zeisel method has shown:

0.2790 gave 0.2886 AgI . $\text{MeO}=13.6$.

$\text{C}_{22}\text{H}_{14}\text{O}_6\text{N}_2(\text{OMe})_2$ requires $\text{MeO}=13.4$ per cent.

Casimiroine is a very weak base, since it is precipitated from its solution in concentrated hydrochloric acid by dilution with water. The only crystalline salts that could be obtained were the *picrate* and the *aurichloride*. The picrate, which was very sparingly soluble, crystallised in small, yellow needles, melting at 165° .

Casimiroine Aurichloride, $\text{C}_{24}\text{H}_{20}\text{O}_8\text{N}_2, \text{HAuCl}_4$.—This salt was prepared by adding a solution of gold chloride to a dilute solution of casimiroine in aqueous alcohol containing a little hydrochloric acid. The yellow precipitate thus produced was crystallised from alcohol, when the salt was obtained in handsome, orange-yellow needles, melting at 195 — 196° :

0.1508 gave 0.1998 CO_2 and 0.0460 H_2O . $\text{C}=36.1$; $\text{H}=3.4$.

0.1207 ,, , on ignition, 0.0296 Au . $\text{Au}=24.5$.

$\text{C}_{24}\text{H}_{20}\text{O}_8\text{N}_2, \text{HAuCl}_4$ requires $\text{C}=35.8$; $\text{H}=2.6$; $\text{Au}=24.5$ per cent.

A solution of the alkaloid in chloroform was found to be devoid of optical activity.

When a little casimiroine, on a porcelain tile, is moistened with a drop of concentrated nitric acid, a yellow colour is first produced, which rapidly deepens to orange-red. With concentrated sulphuric acid the alkaloid, when similarly treated, develops a pale green colour, which, on the addition of a trace of nitric acid, immediately changes to a brilliant orange hue.

Hydrolysis of Casimiroine.

Formation of a New Base, Casimiroitine, $\text{C}_{23}\text{H}_{22}\text{O}_7\text{N}_2$.

On boiling casimiroine for several hours with an alcoholic solution of potassium hydroxide, and then adding a small quantity of water, long, glistening, hair-like needles separate from the liquid

on cooling. By the careful fractional crystallisation of this product from ethyl acetate it was observed not to be homogeneous, but to consist mainly of a substance which separated in stout prisms, melting at 171° , together with a small quantity of a substance which crystallised in fan-like tufts of slender needles, melting at 159° . Only the first-mentioned substance (m. p. 171°) was obtained in an amount sufficient for analysis. On recrystallising this from dilute alcohol, it was obtained, like the original product, in long, glistening, hair-like needles, which formed a felty mass, greatly resembling glass-wool:

0.2680, dried at 110° to a constant weight, lost 0.0188 H_2O .
 $H_2O = 7.0$.

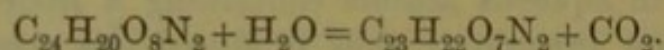
0.1106 * gave 0.2561 CO_2 and 0.0540 H_2O . C = 63.1; H = 5.4.

0.1560 * ,, 8.6 c.c. N_2 at 12.5° and 774 mm. N = 6.6.

$C_{23}H_{22}O_7N_2$ requires C = 63.0; H = 5.0; N = 6.4 per cent.

$C_{23}H_{22}O_7N_2, 2H_2O$ requires $H_2O = 7.6$ per cent.

The above-described hydrolytic product is thus shown to possess the formula $C_{23}H_{22}O_7N_2$, and, being a new compound, it may be designated *casimiroitine*. It appears to have been formed from casimiroine by the addition of a molecule of water and the elimination of a molecule of carbon dioxide, according to the following equation:



When casimiroine is boiled for a few minutes with concentrated hydrochloric acid, it yields an acid which is very sparingly soluble in alcohol and water, and melts above 300° . The amount of material available did not permit of the further study of this reaction.

Isolation of Benzoic Acid.

The ammonium carbonate solution, from which the suspended alkaloid, casimiroine, had been removed by filtration, as already described, was acidified, when a quantity of dark, resinous material was precipitated. As nothing crystalline could be directly isolated from this product, it was dissolved in methyl alcohol, and dry hydrogen chloride passed into the hot solution. The esterified mixture was then poured into water and extracted with ether, the ethereal liquid being subsequently shaken with a dilute solution of sodium hydroxide. The non-phenolic portion of the product possessed the odour of methyl benzoate, and on hydrolysis yielded a small amount of an acid (m. p. 120°), which was identified as benzoic acid. The phenolic portion contained a substance which

* Anhydrous substance.

gave an intense violet coloration with ferric chloride, and was evidently salicylic acid, but the amount of the latter was too small to permit of its isolation.

The original ethereal extract of the aqueous liquid, which had been shaken with a solution of ammonium carbonate, as previously described, was subsequently extracted successively with aqueous sodium carbonate and sodium hydroxide. These alkalis, however, removed only dark-coloured, resinous material, and on finally evaporating the ethereal liquid it left but a slight, oily residue.

Isolation of a New Alkaloid, Casimiroedine, C₁₇H₂₄O₅N₂.

The original aqueous liquid, which had been completely extracted with ether, was next shaken repeatedly with hot amyl alcohol. A portion of the amyl-alcoholic extract was concentrated under diminished pressure to a small bulk, when, on cooling, a quantity of dark-coloured, solid material separated. On treating this with a small amount of cold alcohol, most of the colouring matter was removed, leaving a slightly coloured, apparently crystalline substance. After the separation of the latter, no other crystalline substance could be isolated from the dark-coloured material, even after heating it with 5 per cent. sulphuric acid. As the above-mentioned substance was readily soluble in dilute acids, its isolation was found to be most easily effected by shaking the amyl-alcoholic extract with aqueous 2 per cent. sulphuric acid until the acid liquid ceased to give a precipitate with an aqueous solution of iodine in potassium iodide, and the larger remaining portion of the extract was therefore treated in this manner. The sulphuric acid was subsequently removed from the aqueous liquid by means of barium hydroxide, and the excess of the latter by carbon dioxide, after which the liquid was filtered and concentrated. During the process of evaporation the substance separated in the form of crusts, and the total amount so obtained was 16 grams. The substance, which was found to contain nitrogen, was recrystallised from alcohol, when it separated in wart-like aggregates of very small needles, melting at 222—223°:

0.1613 gave 0.3587 CO₂ and 0.0980 H₂O. C=60.6; H=6.8.

0.1466 „ 10.8 c.c. N₂ at 11° and 743 mm. N=8.6.

C₁₇H₂₄O₅N₂ requires C=60.7; H=7.1; N=8.3 per cent.

The above-described substance, which has thus been shown to be an alkaloid, evidently possesses the formula C₁₇H₂₄O₅N₂. As it is a new compound, it is proposed to designate it *casimiroedine*.

Casimiroedine is a fairly strong base, since it dissolves readily

in dilute acids, and on rendering the solution alkaline the alkaloid only slowly separates in the form of rosettes of needles. It is very sparingly soluble in chloroform, ether, benzene, ethyl acetate, or cold alcohol, but is readily soluble in hot alcohol, and amyl alcohol dissolves it freely.

A slightly acidified solution of casimiroedine, even when exceedingly dilute, gives a reddish-brown precipitate with a solution of iodine in potassium iodide, but potassium-mercuric iodide and picric acid only yield precipitates with a somewhat concentrated solution.

The alkaloid in acid solution was found to be optically active, and a determination of its rotatory power gave the following result:

0.4198, made up to 20 c.c. with 1 per cent. hydrochloric acid, gave $\alpha_D - 1^{\circ}32'$ in a 2-dcm. tube, whence $[\alpha]_D - 36.5^{\circ}$.

Casimiroedine was found to contain no methoxyl group, and in its chemical behaviour is a very indifferent substance. No crystalline derivative could be prepared from it with the exception of the *aurichloride*.

Casimiroedine Aurichloride, $C_{17}H_{24}O_5N_2, HAuCl_4, 2H_2O$.—On the addition of a concentrated solution of gold chloride to a fairly strong solution of casimiroedine in hydrochloric acid the aurichloride is precipitated as an oil, which crystallises on stirring. This salt is dissociated in contact with water, but could be crystallised from 20 per cent. aqueous hydrochloric acid, when it separated in bright yellow, microscopic needles, which melt indefinitely at about 90° in their water of crystallisation. After drying at 70° , however, the salt melts and completely decomposes at $145-148^{\circ}$:

0.0984, dried at 100° to a constant weight, lost 0.0054 H_2O .
 $H_2O = 5.5$.

0.0930 (dried salt) gave, on ignition, 0.0272 Au. $Au = 29.2$.

$C_{17}H_{24}O_5N_2, HAuCl_4, 2H_2O$ requires $H_2O = 5.1$ per cent.

$C_{17}H_{24}O_5N_2, HAuCl_4$ requires $Au = 29.2$ per cent.

The original aqueous liquid, which had been completely extracted with both ether and amyl alcohol, as already described, was subsequently treated with a slight excess of solution of basic lead acetate. A voluminous, light brown precipitate was thus produced, which was collected, thoroughly washed, and decomposed with hydrogen sulphide, but it yielded nothing definite. The filtrate from the basic lead acetate precipitate was treated with hydrogen sulphide for the removal of the excess of lead, and the filtered liquid concentrated under diminished pressure. It then formed a viscid syrup, which contained a considerable quantity of sugar,

since it readily yielded *d*-phenylglucosazone, melting at 205—207°. A portion of the syrup was heated for two hours with dilute sulphuric acid in a reflux apparatus, and then distilled in a current of steam. The distillate contained traces of formic and acetic acids, and on extracting the liquid remaining in the distillation flask with ether, only a further small amount of these acids was obtained. The aqueous liquid was finally treated with barium hydroxide for the removal of the sulphuric acid, filtered, and concentrated, but no crystalline product separated, even after keeping for a considerable time. There was therefore no evidence that the original aqueous liquid contained any substance of a glucosidic nature.

Examination of the Resin (B).

The resinous material, representing that portion of the alcoholic extract of the kernels of the seed which was insoluble in water, formed a soft, oily mass, and amounted to about 375 grams. It was dissolved in alcohol, mixed with purified sawdust, and the thoroughly dried mixture extracted successively in a Soxhlet apparatus with light petroleum (b. p. 35—50°), ether, chloroform, ethyl acetate, and alcohol.

Petroleum Extract of the Resin.

This extract, after the complete removal of the solvent, was a thin, dark-coloured, oily product, amounting to 256 grams. It was digested with 1 litre of ether, when 3·2 grams of a white, apparently crystalline substance remained undissolved, and were collected. This was resolved by fractional crystallisation from alcohol into a substance which was obtained in the form of needles, melting at 196—197°, and amounting to 1·5 grams, and a substance which melted at 232—233°. The first-mentioned substance consisted of the previously described alkaloid, casimiroine, which had been isolated from the aqueous liquid, whilst the second substance was subsequently obtained in much larger amount from the ethereal extract of the resin, and will be further considered in connexion with the latter.

After the separation of the above-mentioned crystalline substances, the clear ethereal solution of the petroleum extract was shaken with aqueous ammonium carbonate, which, however, removed only traces of resinous material. On subsequently attempting to extract the ethereal liquid with sodium carbonate and sodium hydroxide, intractable emulsions were formed. The ether was therefore removed, and the residue hydrolysed by heating with a

solution of 90 grams of potassium hydroxide in one litre of alcohol. After the removal of the alcohol, water was added, and the alkaline liquid repeatedly extracted with ether.

Isolation of Sitosterol, $C_{27}H_{46}O, H_2O$.

The ethereal extract of the alkaline liquid was dried, and the solvent evaporated. A pale yellow, crystalline product was thus obtained, which was dissolved in hot alcohol, when, on cooling, a quantity (8.8 grams) of a substance separated in colourless plates. On recrystallising the substance from a mixture of ethyl acetate and dilute alcohol, it was obtained in needles, melting at $134-135^\circ$:

0.2263, on heating at 100° , lost 0.0114 H_2O . $H_2O=5.0$.

0.1048 * gave 0.3218 CO_2 and 0.1153 H_2O . $C=83.7$; $H=12.2$.

$C_{27}H_{46}O, H_2O$ requires $H_2O=4.5$ per cent.

$C_{27}H_{46}O$ requires $C=83.9$; $H=11.9$ per cent.

The substance was thus identified as a phytosterol, and it gave the colour reactions of that class of compounds.

A determination of its optical rotatory power gave the following result:

0.3118,* made up to 20 c.c. with chloroform, gave $\alpha_D -1^\circ 0'$ in a 2-dcm. tube, whence $[\alpha]_D -32.1^\circ$.

The phytosterol gave an acetyl derivative, which separated from acetic anhydride in small needles, melting at $126-127^\circ$. Its identity with sitosterol was therefore established.

The original alcoholic mother liquor remaining from the crystallisation of the sitosterol was evaporated, when a quantity (10 grams) of viscid, oily material was obtained. This was found to consist chiefly of oxygenated substances, but nothing definite could be isolated from it.

Isolation of Ipuranol, $C_{23}H_{38}O_2(OH)_2$.

The alkaline liquid, from which the sitosterol had been removed by extraction with ether, was acidified, and the liberated fatty acids taken up with ether. The ethereal liquid contained a quantity of a dark-coloured, crystalline substance in suspension. This was collected, heated with alcohol for the removal of colouring matter, and then recrystallised from dilute pyridine, from which it separated in microscopic needles, melting at $280-285^\circ$. The amount of substance so obtained was 2.9 grams. (Found, $C=72.6$; $H=10.4$. Calc., $C=72.6$; $H=10.5$ per cent.)

* Anhydrous substance.

The above results, together with the colour reactions yielded by this substance, established its identity as ipuranol.

When heated with acetic anhydride it gave diacetylipuranol, which separated from alcohol in shining leaflets, melting at 166° . (Found, C=69.7; H=9.5. Calc., C=69.8; H=9.5 per cent.)

On heating the ipuranol, in pyridine solution, with benzoyl chloride, a *dibenzoyl* derivative was obtained, which, after crystallisation from a mixture of ethyl acetate and alcohol, separated in needles melting at 197° .

A determination of the optical rotatory power of the ipuranol gave the following result:

0.1708, made up to 20 c.c. with pyridine, gave $\alpha_D - 0^{\circ}41'$ in a 2-dcm. tube, whence $[\alpha]_D - 40.0^{\circ}$.

Identification of the Fatty Acids.

The ethereal liquid from which the ipuranol had been removed, as above described, was evaporated to a low bulk, and a large quantity of light petroleum added. This precipitated some resinous material, which was separated, after which the liquid was again evaporated. A quantity (105 grams) of fatty acids was thus obtained, which, when distilled under diminished pressure, passed over between 200° and $230^{\circ}/12$ mm.

In order to effect a separation of the solid and liquid acids, a portion (25 grams) of the mixture was converted into the lead salts, and the latter digested with ether, when the greater part dissolved. Both the soluble and insoluble portions were decomposed by hydrochloric acid, and the liberated fatty acids separately examined.

The Liquid Acids.—These acids, when distilled under diminished pressure, passed over between 235° and $250^{\circ}/15$ mm. as a yellow oil. An analysis and determination of the constants gave the following results:

0.1026 gave 0.2906 CO_2 and 0.1099 H_2O . C=77.2; H=11.9.

0.2646 absorbed 0.4120 iodine. Iodine value=155.7.

0.3094 neutralised 0.0621 KOH. Neutralisation value=200.7.

$\text{C}_{18}\text{H}_{34}\text{O}_2$ requires C=76.6; H=12.1 per cent. I.V.=90.1;
N.V.=198.9.

$\text{C}_{18}\text{H}_{32}\text{O}_2$ requires C=77.1; H=11.4 per cent. I.V.=181.4;
N.V.=200.4.

$\text{C}_{18}\text{H}_{30}\text{O}_2$ requires C=77.7; H=10.8 per cent. I.V.=274.1;
N.V.=201.8.

These results would indicate that the liquid acids consist of a mixture of oleic, linolic, and linolenic acids.

The Solid Acids.—These acids, when crystallised from glacial acetic acid, separated in glistening leaflets, melting at 60—62°:

0.1030 gave 0.2875 CO₂ and 0.1194 H₂O. C=76.1; H=12.9.

0.2292 neutralised 0.0461 KOH. Neutralisation value=201.1.

C₁₆H₃₂O₂ requires C=75.0; H=12.5 per cent. N.V.=219.1.

C₁₈H₃₆O₂ „ C=76.1; H=12.7 „ „ N.V.=197.5.

The solid acids would thus appear to consist of a mixture of palmitic and stearic acids, the latter predominating.

Ethereal Extract of the Resin.

This extract, after the removal of the solvent, formed a brittle mass, which could readily be reduced to a light brown powder, and amounted to 75 grams. It was heated with 2 litres of alcohol, when the greater portion dissolved. The small insoluble portion (2.3 grams) was collected, and identified as ipuranol.

Isolation of a New Lactone, Casimirolid, C₂₄H₂₈O₆.

The above-mentioned hot alcoholic liquid deposited on cooling a quantity (22.1 grams) of well-developed crystals, which, when recrystallised from alcohol with the addition of a little animal charcoal, separated in colourless prisms, melting at 229—230°:

0.0960 gave 0.2448 CO₂ and 0.0594 H₂O. C=69.5; H=6.9.

0.1326, in 21.16 benzene, gave Δt —0.072°. M.W.=426.

C₂₄H₂₈O₆ requires C=69.9; H=6.8 per cent. M.W.=412.

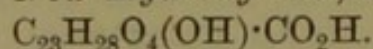
From these results it is evident that the above-described substance possesses the formula C₂₄H₂₈O₆, and that it is a new compound. Having ascertained that it is a lactone, as shown below, it is proposed to designate it *casimirolid*.

Casimirolid is readily soluble in ether, chloroform, and ethyl acetate. It is optically active, and a determination of its specific rotatory power gave the following result:

0.3856, made up to 25 c.c. with chloroform, gave α_D —1°31' in a 2-dcm. tube, whence $[\alpha]_D$ —49.2°.

Hydrolysis of the Lactone.

Formation of a New Hydroxy-acid, Casimiroic Acid,



When casimirolid is shaken with a cold solution of potassium hydroxide in aqueous alcohol, it readily takes up a molecule of water, and is converted into an acid, which is precipitated on acidifying the alkaline liquid. This acid crystallises very readily from alcohol in rosettes of glistening needles, melting at 207°:

0.1074 gave 0.2638 CO_2 and 0.0698 H_2O . $\text{C} = 67.0$; $\text{H} = 7.2$.
 0.2792 neutralised 0.0359 KOH . M.W. (monobasic acid) = 436.

$\text{C}_{24}\text{H}_{30}\text{O}_7$ requires $\text{C} = 67.0$; $\text{H} = 7.0$ per cent. M.W. = 430.

The above-described acid is thus shown to possess the formula $\text{C}_{24}\text{H}_{30}\text{O}_7$. Like the corresponding lactone, it is a new compound, and it is proposed to designate it *casimiroic acid*.

A determination of the optical rotatory power of the acid gave the following result:

0.1670, made up to 20 c.c. with absolute alcohol, gave $\alpha_D - 1.27'$ in a 2-dcm. tube, whence $[\alpha]_D - 86.8^\circ$.

Casimiroic acid, unlike the lactone, is very sparingly soluble in ether, chloroform, and ethyl acetate. Its sodium salt is very readily soluble in water and in alcohol. On the addition of a solution of copper sulphate to a neutral solution of the sodium salt, a pale blue, amorphous precipitate of copper casimiroate was produced, the analysis of which, however, indicated it to consist of a basic salt.

Silver Casimiroate, $\text{C}_{23}\text{H}_{29}\text{O}_5 \cdot \text{CO}_2\text{Ag}$.—This salt was obtained as a white, amorphous precipitate on the addition of a solution of silver nitrate to a solution of the sodium salt. It is sparingly soluble in water, and gradually darkens on exposure to light:

0.1435 of salt gave, on ignition, 0.0296 Ag . $\text{Ag} = 20.6$.

$\text{C}_{24}\text{H}_{29}\text{O}_7\text{Ag}$ requires $\text{Ag} = 20.1$ per cent.

Methyl Casimiroate, $\text{C}_{23}\text{H}_{29}\text{O}_5 \cdot \text{CO}_2 \cdot \text{CH}_3$.—This ester was prepared by passing dry hydrogen chloride into a solution of the acid in methyl alcohol at the boiling temperature. It was very soluble in alcohol, and could only be obtained with difficulty in a crystalline state from dilute alcohol, when it melted at $108-110^\circ$:

0.1086 gave 0.2666 CO_2 and 0.0739 H_2O . $\text{C} = 66.9$; $\text{H} = 7.6$.

$\text{C}_{25}\text{H}_{32}\text{O}_7$ requires $\text{C} = 67.5$; $\text{H} = 7.2$ per cent.

Acetylcasimiroic Acid, $\text{C}_{23}\text{H}_{28}\text{O}_4(\text{O} \cdot \text{CO} \cdot \text{CH}_3) \cdot \text{CO}_2\text{H}$.—This was obtained by heating casimiroic acid with acetic anhydride, pouring the product into water, and digesting the resulting precipitate with dilute aqueous sodium carbonate, when, on acidifying the alkaline liquid, the *acetyl* derivative was precipitated. It was exceedingly soluble in the usual organic solvents, but could be obtained from dilute alcohol in colourless, microscopic needles. The acetyl derivative has no sharp melting point, but gradually decomposes at temperatures above 142° :

0.1149 gave 0.2774 CO_2 and 0.0738 H_2O . $\text{C} = 65.8$; $\text{H} = 7.1$.

$\text{C}_{26}\text{H}_{32}\text{O}_8$ requires $\text{C} = 65.8$; $\text{H} = 6.8$ per cent.

Isolation of a Yellow Phenolic Substance, C₁₆H₁₂O₆.

After the separation of the lactone, casimiroid, from the alcoholic solution of the ethereal extract of the resin, as previously described, the solvent was removed, and the residue, which still contained a small amount of lactone, dissolved in ether. The ethereal liquid was then successively extracted with solutions of ammonium carbonate, sodium carbonate, and sodium hydroxide. The ammonium carbonate removed only a small amount of resinous substance, from which nothing definite could be isolated. The sodium carbonate extract also consisted chiefly of resinous material, but by digesting the latter with alcohol, in which the resin was very soluble, a small amount (0.15 gram) of a crystalline substance was isolated. This substance, when recrystallised from dilute alcohol, separated in small, yellow needles, melting at 215—218°. Its solution gave a yellow colour with alkalis and with ferric chloride. With concentrated sulphuric acid it yielded an orange-red solution, slowly changing to pale yellow, but showing no fluorescence:

0.0816 gave 0.1904 CO₂ and 0.0290 H₂O. C=63.6; H=3.9.

C₁₆H₁₂O₆ requires C=64.0; H=4.0 per cent.

The *substance* thus appears to possess the formula C₁₆H₁₂O₆, but the amount available did not permit of its further characterisation. Although isomeric with the flavone derivatives, kaempferid (*Ber.*, 1881, **14**, 2385) and the luteolin monomethyl ether prepared synthetically by Diller and Kostanecki (*Ber.*, 1901, **34**, 1452), it is not identical with either of these compounds.

The sodium hydroxide extract of the above-mentioned ethereal liquid yielded on acidification a quantity (4.2 grams) of a crystalline substance, which, when recrystallised from alcohol, separated in glistening needles, melting at 207°. This substance was identified as the previously described casimiroic acid, which had evidently been formed by the action of the alkali on the lactone present in the ethereal liquid. After the successive extraction of the ethereal liquid with the above-mentioned alkalis, the solvent was evaporated, but only a slight, oily residue remained.

Chloroform, Ethyl Acetate, and Alcohol Extracts of the Resin.

The chloroform extract of the resin formed a hard, black mass, and amounted to 18.6 grams. The only crystalline substance obtained from it was a small quantity (0.6 gram) of the previously described alkaloid, casimiroine. The ethyl acetate and alcohol

extracts were dark-coloured products, and amounted to 3.2 and 22 grams respectively. Both of these extracts were heated with 5 per cent. sulphuric acid in aqueous alcohol, but they yielded nothing definite, although the aqueous liquid resulting from this treatment of the alcohol extract evidently contained some sugar, for it readily reduced Fehling's solution, and from the liquid, after the removal of the sulphuric acid, a little *d*-phenylglucosazone (m. p. 206°) was prepared.

II.—*Examination of the Shells.*

A small portion (10 grams) of the ground shells was subjected to a preliminary test for an alkaloid by digestion with Prollius' fluid. The reactions obtained were much less marked than in the case of the kernels, but indicated the presence of a small amount of alkaloidal substance.

Another portion (50 grams) of the ground material was successively extracted with various solvents, when the following amounts of extract, dried at 100°, were obtained:

Petroleum (b.p. 35—50°)	extracted	0.2 gram	=	0.4 per cent.
Ether	"	0.3 "	"	0.6 "
Chloroform	"	0.1 "	"	0.2 "
Ethyl acetate	"	0.1 "	"	0.2 "
Alcohol	"	0.8 "	"	1.6 "
		—		—
Total.....		1.5 grams	=	3.0 per cent.

For a complete examination of the shells a quantity (10.39 kilograms) of the ground material was completely extracted with hot alcohol. After the removal of the greater portion of the solvent, a thin extract, amounting to 482 grams, was obtained.

The whole of the above-mentioned extract was first distilled in a current of steam, when it yielded a very small amount (0.65 gram) of an essential oil. This oil appeared to be similar in character to that obtained from the kernels of the seed, but the amount was not sufficient for its further examination.

The aqueous liquid and resinous material remaining after the above-described treatment of the extract were examined by methods similar to those described in connexion with the kernels of the seed.

The chief constituents of the aqueous liquid were found to be amorphous colouring matter and sugar, the latter yielding *d*-phenylglucosazone, melting at 206°. No other crystalline product could be obtained from the liquid, the amount of alkaloidal substance present having proved to be too small to permit of its isolation.

The resinous material, when thoroughly washed and dried, formed a light brown, friable mass, and amounted to 95 grams, or about 0.9 per cent. of the weight of shells employed. The resin was

successively extracted with various solvents, and the resulting products separately examined. The petroleum extract, which amounted to 37 grams, contained a considerable quantity (20 grams) of stearic acid in a free state, together with a little free palmitic acid, and a small amount of these acids in the form of glycerides. The stearic acid, when crystallised from alcohol, separated in pearly leaflets, melting at 67—68°. (Found, C=76.2; H=13.1. Calc., C=76.1; H=12.7 per cent.) The ether, chloroform, ethyl acetate, and alcohol extracts of the resin amounted to 8.8, 30.4, 1.5, and 14.5 grams respectively, and consisted entirely of amorphous products. The chloroform and alcohol extracts were heated with 5 per cent. sulphuric acid in aqueous alcohol, but as they yielded no sugar by this treatment, they were not glucosidic in character.

Physiological Tests.

In order to ascertain whether either the hypnotic or toxic action attributed to *Casimiroa* seed (*loc. cit.*) could be confirmed, a number of tests were kindly conducted for us at the Wellcome Physiological Research Laboratories by Drs. H. H. Dale and P. P. Laidlaw, to whom our best thanks may here be expressed. All the products, with the exception of the essential oil, as noted below, were administered to dogs by the mouth.

The finely-divided kernel of an entire seed, weighing about 2 grams, as also 1 gram of the dry enzyme or protein material obtained from the seed, and representing 90 grams of the kernel, had no effect. One gram of the alcoholic extract, representing 3.2 grams of the kernels of the seed, produced vomiting and a temporary depression, which was apparently due to the repeated emesis, but no other effect could be observed. A similar effect was produced by the administration of 1 gram each of the petroleum and alcohol extracts of the resin, whereas the ether, chloroform, and ethyl acetate extracts of the resin were inactive. The following crystalline substances which had been isolated from the seed, namely, casimiroine, $C_{24}H_{20}O_8N_2$, casimiroedine, $C_{17}H_{24}O_5N_2$, and the lactone, casimiroolid, $C_{24}H_{28}O_6$, were given to dogs in amounts of 0.4 gram, and also to cats in doses of 0.1 gram respectively, but the animals remained perfectly normal. Inasmuch as the experiments of Chevalier (*loc. cit.*) led him to conclude that the therapeutic value of *Casimiroa* seed is due to the essential oil or the resin which he presumed to be produced therefrom, a special test was conducted with the essential oil. A quantity (0.25 c.c.) of this product representing about 1100 grams of the kernels of the seed, when given to a cat, produced salivation and slight depression,

which may be ascribed to the nauseating taste of the substance, but there was no further effect, and particularly no trace of narcosis.

Summary.

The material employed for this investigation consisted of the fresh seeds of *Casimiroa edulis*, La Llave and Lejarza (Nat. Ord. *Rutaceae*), which were obtained directly from Mexico.

For the purpose of their examination, the kernels of the seed were first separated from the dry, fibrous shells. The latter, which represented about one-fifth the weight of the entire fresh seed, were separately examined, but yielded little of interest. They were found to contain a small amount of an essential oil, traces of alkaloidal substance, some sugar, and considerable stearic acid in the free state, together with resinous material.

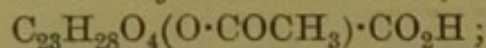
The kernels of the seed, when subjected to a preliminary test, gave reactions indicating the presence of a considerable proportion of an alkaloid. They were also found to contain an enzyme, which was obtained in the form of a light brown powder, and slowly effected the hydrolysis of amygdalin.

An alcoholic extract of the ground kernels, when distilled in a current of steam, yielded a small amount of a pale yellow essential oil, which gradually darkened in colour. This oil had an agreeable, aromatic odour, and the following constants: $d = 0.9574$ at 20° ; $\alpha_D + 2^\circ 25'$ in a 25 mm. tube.

From the portion of the extract which was soluble in water there were isolated: (i) a new alkaloid, *casimiroine*, $C_{24}H_{20}O_8N_2$ (m. p. $196-197^\circ$), which is a very weak base, but yields a crystalline *picrate* (m. p. 165°) and an *aurichloride* (m. p. $195-196^\circ$). On heating *casimiroine* with alkalis, it undergoes hydrolysis, with the elimination of carbon dioxide, yielding a new base, *casimiroitine*, $C_{23}H_{22}O_7N_2$ (m. p. 171°), together with a small amount of a basic substance melting at 159° ; (ii) a new alkaloid, *casimiroedine*, $C_{17}H_{24}O_5N_2$ (m. p. $222-223^\circ$; $[\alpha]_D$, in acid solution, -36.5°), which yields a crystalline *aurichloride*; (iii) benzoic acid, with apparently a trace of salicylic acid. The aqueous liquid contained, furthermore, a quantity of sugar, which yielded *d*-phenylglucosazone (m. p. $205-207^\circ$).

The portion of the alcoholic extract which was insoluble in water consisted of a soft, oily resin. From this material the following compounds were isolated: (i) sitosterol, $C_{27}H_{46}O$ (m. p. $134-135^\circ$; $[\alpha]_D - 32.1^\circ$), (ii) ipuranol, $C_{23}H_{38}O_2(OH)_2$; (iii) a mixture of fatty acids, consisting of palmitic, stearic, oleic, linolic, and linolenic acids; (iv) a new lactone, *casimirolid*, $C_{24}H_{28}O_6$ (m. p. $229-230^\circ$; $[\alpha]_D - 49.2^\circ$), which yields a new hydroxy-acid, designated as

casimiroic acid, $C_{23}H_{28}O_4(OH) \cdot CO_2H$ (m. p. 207° ; $[\alpha]_D - 86.8^\circ$). The following derivatives of this acid were prepared: *silver casimiroate*, $C_{23}H_{29}O_5 \cdot CO_2Ag$; *methyl casimiroate*, $C_{23}H_{29}O_5 \cdot CO_2CH_3$ (m. p. $108-110^\circ$); and *acetylcasimiroic acid*,



(v) a yellow, phenolic *substance*, $C_{16}H_{12}O_6$ (m. p. $215-218^\circ$).

It may finally be noted that the results of the present investigation of *Casimiroa* seed have afforded no evidence of the presence of a definite glucoside or a so-called "glucoalkaloid," as has previously been affirmed, and physiological tests conducted with animals have likewise failed to confirm their reputed hypnotic or toxic properties.

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