

[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY, FACULTAD DE CIENCIAS EXACTAS, FÍSICAS Y NATURALES, UNIVERSITY OF BUENOS AIRES AND THE INSTITUTO BACTERIOLÓGICO D.N.H.]

Studies on Argentine Plants. V.¹ Identification and Characterization of Some Alkaloids in *Fagara Coco* (Gill) Engl.

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In 1925 Stuckert² and his collaborators undertook the isolation of alkaloids from the leaves and young twigs of *Fagara coco* (Gill) Engl., a tree growing in central and northern Argentina and in Bolivia, and which apparently has been used in those regions as a drug. They obtained several crystalline substances of which the three best characterized were named and described as follows: α -fagarine, $C_{18}H_{22}NO_4$, m. p. 169°; β -fagarine, $C_{12}H_{26}NO_6$, m. p. 176°; and γ -fagarine, $C_{15}H_{16}NO_3$, m. p. 139°. At Stuckert's request, Merck and Company (Darmstadt) carried out a large-scale preparation of alkaloids from *Fagara coco* and isolated: fagarine I, melting at 163°, $C_{19}H_{23}NO_5$ which is probably identical with Stuckert's α -fagarine, and fagarine II, apparently new, which melted at 202° and had a composition corresponding to $C_{18}H_{20}NO_4$.

By extraction and purification of the alkaloids from the end twigs and leaves of a sample of *Fagara coco* kindly supplied to us by Dr. Stuckert, we have obtained three well-defined substances corresponding to the α , β and γ -fagarines mentioned above. Revised formulas and melting points are given in the experimental part. In addition, small fractions of a higher-melting material which may correspond to Merck fagarine II have been obtained, but not in sufficient quantity for complete purification.

Our investigation of the properties of these alkaloids shows that two of them bear a very close relationship to substances already described in the literature. β -Fagarine is identical with skimmianine which was discovered by Honda³ in *Skimmia japonica*, a plant belonging like *Fagara* to the family of Rutaceae. The structure of skimmianine has been established by Asahina and Inubuse⁴ who showed that this alkaloid is a dimethoxy derivative of *dictamine* which was found by Thoms⁵ in *Dictamus albus* and by Asa-

hina, Ohta and Inubuse⁶ in *Skimmia repens*. The identity of β -fagarine and skimmianine is proved by the close correspondence of their melting points and the melting points of a series of six derivatives and degradation products which were prepared from β -fagarine by the present authors according to the method originally described by Asahina for skimmianine.

COMPARISON OF PROPERTIES

| Substance | Melting points, °C. | |
|---|------------------------|-------------------------------|
| | From β -fagarine | From skimmianine ⁴ |
| Free base, $C_{14}H_{13}NO_4$ | 178 | 176 |
| Picrate of base, $C_{14}H_{13}NO_4 \cdot C_6H_4N_3O_6$ | 195 | 195-197 |
| Iso-skimmianine, $C_{14}H_{13}NO_4$ | 188-9 | 185 |
| Dimethoxy-isoskimmianine | 218-220 | 218 |
| Skimmianic acid | 248 | 248 |
| Skimmianal | 238 | 238 |
| 2,4-Dihydroxy-7,8-dimethoxyquinoline | 250 | 250 |

It is recommended that the name β -fagarine be eliminated and the name skimmianine be used henceforth.

γ -Fagarine apparently has not been described under another name and the retention of the present term is therefore recommended. Nevertheless, this alkaloid is very similar to skimmianine, from which it differs by having one less methoxyl group. It forms a series of derivatives and degradation products entirely analogous to those described for skimmianine but not identical. On oxidation by permanganate, γ -fagarine yields γ -fagaric aldehyde and γ -fagaric acid. The latter, which also is derived from the aldehyde by further oxidation, on decarboxylation yields a 2,4-dihydroxymethoxyquinoline in which the position of the methoxyl group is unknown. Since none of the four isomeric dihydroxymethoxyquinolines has yet been synthesized, we have begun their synthesis in order to make these key reference compounds available for direct comparisons.

α -Fagarine is a substance quite different from these compounds and since it has not been de-

(1) Part IV, R. A. Gentile and R. Labriola, *J. Org. Chem.*, **7**, 136 (1942).

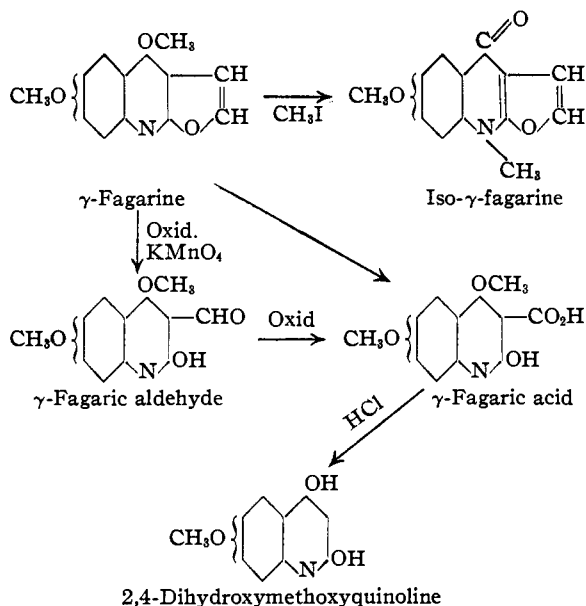
(2) "Investigaciones del Laboratorio de Química Biológica, Córdoba, Argentina," Vol. I, 1933, and Vol. II, 1938.

(3) Honda, *Arch. Exp. Path. Pharmacol.*, **52**, 83 (1904).

(4) Asahina and Inubuse, *Ber.*, **63**, 2052 (1930).

(5) Thoms, *Ber. Deut. Pharm. Ges.*, **33**, 68 (1923); Thoms and Dambergis, *Arch. Pharm.*, **265**, 39 (1930).

(6) Asahina, Ohta and Inubuse, *Ber.*, **63**, 2045 (1930).



scribed under another name as far as we know, we recommend retaining the present term. We have assigned to it the formula $\text{C}_{18}\text{H}_{21}\text{NO}_4$. Of the four oxygens, two belong to the methoxyl groups. It possesses a methylimino group and is a stronger base than skimmianine or γ -fagarine from which it may be separated easily by exploiting this difference. Preliminary experiments indicate that its behavior with methyl iodide or oxidizing agents is completely different from that of the other two bases. We are continuing its investigation.

Experimental Part

Acid Extraction.—Ten kg. of leaves and twigs of *Fagara coco* was soaked for four days in 60 liters of 10% hydrochloric acid, and this extraction was twice repeated at four-day intervals. On evaporation *in vacuo* to about 10 l., a precipitate formed which was filtered off and freed from alkaloids by repeated maceration with 20% hydrochloric acid. The combined alkaloidal extract was neutralized with dilute sodium hydroxide and extracted with trichloroethylene. The washed extract was evaporated to dryness and the residue was extracted repeatedly with 13% hydrochloric acid. The combined acid extracts were neutralized and extracted with chloroform. The residue from the washed chloroform extract was again extracted with 13% hydrochloric acid. This acid extract was treated with dilute sodium hydroxide until the weaker bases precipitated at pH 3.5–4.0. After twenty-four hours, the precipitate containing skimmianine and γ -fagarine was recrystallized from alcohol yielding skimmianine. Concentration of the mother liquors gave a product, 120–150%, in which γ -fagarine predominates and from which it can be crystallized in large prisms from alcoholic solution.

The alkaline mother liquor was brought to pH 9 with sodium hydroxide and exhaustively extracted with

chloroform. Evaporation of the extract and recrystallization of the residue from alcohol yielded α -fagarine.

Acid extraction of 10 kg. of leaves gave 54 g. of impure alkaloidal residue yielding 13 g. of skimmianine, m. p. 178°; 6 g. of γ -fagarine, m. p. 139°; and 7 g. of α -fagarine, m. p. 163°.

Alcoholic Extraction.—Two kg. of leaves was boiled first with 8 l. of 96% ethanol for four hours and the extraction repeated three times with 5-l. portions of alcohol and, finally, with a 5-l. portion of alcohol to which 250 cc. of concentrated ammonium hydroxide had been added. The combined extracts from 10 kg. of leaves, after neutralizing with hydrochloric acid and drying, gave a residue of about 1.5 kg. After cooling this was extracted with 4 successive portions of cold, dilute hydrochloric acid, the combined extracts neutralized with 30% sodium hydroxide solution and then extracted exhaustively with trichloroethylene. The residue was then extracted with 2 l. of very dilute hydrochloric acid and the combined extracts were rendered alkaline, extracted with chloroform, the product washed with water, dried, and the residue again extracted with very dilute hydrochloric acid. Water was added up to 2 l., a fine suspension removed by filtration, and sodium hydroxide slowly added to pH 2. At this point a precipitate which did not give the alkaloidal reaction was formed and this was removed by filtration. The pH of the solution was brought to 3.5–4 and the procedure was continued as given for the acid extraction. The yield from the 10 kg. of leaves was 41.5 g. of skimmianine, m. p. 178°, 2.5 g. of γ -fagarine, m. p. 139°, and 5 g. of α -fagarine, m. p. 162°.

Characterization of γ -Fagarine as a Methoxy-dictamine.—When γ -fagarine of m. p. 139° was recrystallized several times from alcohol, prismatic crystals of m. p. 142° were obtained, soluble in chloroform, benzene and ether, slightly soluble in petroleum ether, and very slightly soluble in water. Alcohol was the best solvent for recrystallization.

Anal. Calcd. for $\text{C}_{18}\text{H}_{11}\text{NO}_3$: C, 68.12, H, 4.80; N, 6.11; two methoxyl, 27.03. Found: C, 68.14, 68.39; H, 4.99, 5.11; N, 6.22; $-\text{OCH}_3$, 27.12, 27.09.

γ -Fagarine Picrate.—Prepared in the usual way⁴ in alcohol solution and recrystallized from the same solvent, the picrate was obtained in the form of yellow needles of m. p. 177°.

Anal. Calcd. for $\text{C}_{18}\text{H}_{11}\text{NO}_3 \cdot \text{C}_6\text{H}_3\text{N}_3\text{O}_7$: N, 12.22. Found: N, 12.50.

γ -Fagarine Picrolonate.—Prepared in the usual way,⁴ and recrystallized from alcohol, the picrolonate formed yellow needles melting at 174–175°.

Anal. Calcd. for $\text{C}_{18}\text{H}_{11}\text{NO}_3 \cdot \text{C}_{10}\text{H}_8\text{N}_4\text{O}_5$: N, 14.60. Found: N, 14.84.

The Rearrangement of γ -Fagarine to Iso- γ -fagarine.—A sample of 500 mg. of γ -fagarine was treated in a closed tube at 100–105° with 3 ml. of methyl iodide for three hours. When the heating was over, the solution was filtered and evaporated to dryness. The residue thus obtained was crystallized from methanol, after decolorization by carbon. There was obtained 300 mg. of colorless needles, melting at 179°.

Anal. Calcd. for $\text{C}_{18}\text{H}_{11}\text{NO}_3$: C, 68.12; H, 4.80; N, 6.11. Found: C, 67.93; H, 5.18; N, 6.36.

γ -Fagaric Aldehyde.—A sample of 800 mg. was dissolved in 60 ml. of acetone heated almost to the boiling point and a hot acetone solution of 1.6 mg. of potassium permanganate was slowly added. Oxidation occurred easily. After cooling, the manganese dioxide was filtered off, and the solution was evaporated to dryness. The residue (100 mg.), recrystallized from alcohol, yielded fine yellow needles, melting at 185°.

Anal. Calcd. for $C_{12}H_{11}NO_3$: C, 61.80; H, 4.72. Found: C, 61.94; H, 5.08.

γ -Fagaric Aldehyde Phenylhydrazone.—The phenylhydrazone of the preceding aldehyde was prepared by heating the latter in alcohol solution for twenty minutes with a slight excess of phenylhydrazine and acetic acid. Upon addition of water at the end of heating, a precipitate was formed which was filtered off and, when recrystallized from alcohol, yielded yellow needles melting at 207°.

Anal. Calcd. for $C_{18}H_{17}N_3O_3$: N, 13.00. Found: N, 13.37.

γ -Fagaric Acid.—The manganese dioxide precipitate obtained during the oxidation of fagarine was digested with 10% sodium hydrate and filtered. The resultant solution was acidified with hydrochloric acid which yielded a precipitate of γ -fagaric acid. It was quite insoluble in ordinary organic solvents. It recrystallized from a large volume of boiling acetone to which carbon had been added. There was obtained 100 mg. of colorless needles, melting at 215°.

Anal. Calcd. for $C_{12}H_{11}NO_5$: N, 5.62. Found: N, 5.68.

The same acid was obtained by oxidizing with potassium permanganate a hot acetone solution of γ -fagaric aldehyde. The crystals obtained also melted at 215° and were not distinguishable from those obtained by direct oxidation of the base.

2,4-Dihydroxymethoxyquinoline.—A sample of 380 mg. of γ -fagaric acid was suspended in 40 ml. of dilute hydrochloric acid⁷ and boiled until a clear solution was obtained. On cooling, a crystalline precipitate, melting at 225° and weighing 320 mg. was obtained. Recrystallized from alcohol, it yielded long prisms melting at 250°.

Anal. Calcd. for $C_{10}H_9NO_3$: C, 62.82; H, 4.71; N, 7.33. Found: C, 63.10; H, 5.12; N, 7.59.

⁷) Strong acid of density 1.19 diluted 1:2.

Nitrous Derivatives of 2,4-Dihydroxymethoxyquinoline.—An alkaline solution of the quinoline derivative mentioned, containing the necessary amount of sodium nitrite, was poured slowly into a solution of 10% sulfuric acid with stirring. There was obtained a reddish precipitate which was filtered and was recrystallized several times from acetic acid, yielding intensely red needles which melted and decomposed at 216–217°.

Anal. Calcd. for $C_{10}H_9N_2O_4$: N, 12.72. Found: N, 13.02.

α -Fagarine.—The fraction precipitating at pH 9 was purified by repeated recrystallization from alcohol. Two kinds of crystals have been obtained: prismatic, melting at 163°, and octahedral, melting at 169°. Recrystallized from chloroform-petroleum ether, long prisms were obtained, melting at 169°. These are the crystals which were finally analyzed. They were soluble in benzene, chloroform, ether and acetone, slightly so in petroleum ether. When pure, they were slightly soluble in water. The substance has no optical activity.

Anal. Calcd. for $C_{18}H_{21}NO_4$: C, 68.57; H, 6.42; N, 4.44; two methoxyls, 19.68; one $-NCH_3$ group, 9.23. Found: C, 68.58, 68.47; H, 6.36, 6.25; N, 4.33, 4.28; $-OCH_3$, 18.45; $-NCH_3$, 12.80.

α -Fagarine treated for twenty minutes with 40% sulfuric acid and phloroglucinol in a boiling water-bath yielded a red precipitate which, according to Gadamer, is characteristic of dioxymethylene groups.

Summary

The study of the alkaloids in the twigs and leaves of *Fagara coco* (Gill) Engl. has confirmed the presence of those described by Stuckert as α -, β - and γ -fagarines.

β -Fagarine has been identified with skimmianine.

γ -Fagarine has been degraded to a 2,4-dihydroxymethoxyquinoline, and it has been established that it is a methoxydictamine.

α -Fagarine is a base of a different type from the preceding ones. Some of its functional groups have been determined.

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