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STUDIES ON ARGENTINE PLANTS—XXIII[1]

QUATERNARY BASES FROM *COLLETIA SPINOSISSIMA* GMEL.[2]

E. SÁNCHEZ[3] and J. COMIN
Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales
Perú 222, Buenos Aires, Argentina

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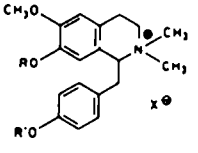
Abstract—From the aerial parts of *Colletia spinosissima* Gmel. (Rhamnaceae), two quaternary benzyltetrahydroisoquinoline alkaloids have been isolated. One, was identified as D-(−)- magnocurarine (I), and the structure of the other, which was named *colletine*, established as D-(−)-1-(4-methoxybenzyl)-2,2-dimethyl-6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (II).

Colletia spinosissima Gmel. is a thorny shrub belonging to the Rhamnaceae family, tribe Colletiae, growing wild in a broad area of Argentina. The use of this plant in indigenous medicine, principally as a febrifuge, is mentioned by several authors.[4] The sample studied was collected near Punta Indio, Provincia de Buenos Aires, Argentina, in July 1963. The methanolic extract of the dried aerial parts of the plant yielded a residue which was taken up in dilute hydrochloric acid. From this solution, a quaternary alkaloid fraction was isolated by ion exchange and fractionated by countercurrent distribution. Two main fractions were further purified by chromatography yielding two quaternary bases as chlorides.

One, was identified as D-(—)-magnocurarine (I) and the other as a new benzyltetrahydroisoquinoline quaternary alkaloid for which we propose the name D-(—)-*colletine* (II).



I: R = R' = H ; $X^{\ominus} = HO^{\ominus}$
II: R = H ; R' = CH_3 ; $X^{\ominus} = Cl^{\ominus}$ or $I^{\ominus}$
III: R = R' = CH_3 ; $X^{\ominus} = I^{\ominus}$

Magnocurarine yielded a picrate, m.p. 185–185·5°, which was transformed into the quaternary base, $C_{19}H_{25}NO_4$, m.p. 200–201°, $[\alpha]_D^{20}$ −97·2° (water). M.p., elemental analysis, rotatory power, picrate m.p., as well as the UV and specially the NMR

[1] Part XXII: H. Pozzi, E. Sánchez and J. Comin, *Tetrahedron* **23**, 1129 (1967).

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[4] J. Hieronymus, *Plantas Diafóricas, Flora Argentina* p. 72. Editorial Atlántida, Buenos Aires; J. A. Domínguez, *Contribuciones a la Materia Médica Argentina* p. 106, Peuser, Buenos Aires (1928).

spectrum (Fig. 1), suggested identity with magnocurarine. This was proved by direct comparison with an authentic sample of L—(+)-magnocurarine: the IR spectra of both the quaternary bases and the picrates were identical. The mixed m.p. of the picrates showed a marked depression: this being due to the fact that both samples were enantiomers and the racemic picrate melts at 174°.[5]

Colletine was obtained as a crystalline chloride that could be recrystallized from ethyl acetate–ethanol, m.p. 130–132°, $[\alpha]_D^{20}$ −132·8° (ethanol). It analysed for the molecular formula $C_{20}H_{26}NO_3Cl \cdot H_2O$, including two MeO groups. The UV spectrum suggested a benzyltetrahydroisoquinoline skeleton, and its bathochromic shift in alkaline solution showed the presence of a phenolic OH group. This was confirmed by an NMR spectrum in DMSO which shows a singlet at $\delta = 9{\cdot}2$ disappearing on addition of D_2O.[6] The NMR spectrum in trifluoroacetic acid (Fig. 1) was very similar to the spectra of other quaternary benzyltetrahydroisoquinoline alkaloids with three oxygenated substituents (coclaurine-type).[7]

To confirm the substitution pattern, colletine chloride was transformed by ion exchange into the iodide, $C_{20}H_{26}NO_3I$, m.p. 169–173°, which on treatment with MeI in methanolic KOH gave the iodide of O-methylcolletine, m.p. 136·5–138°. This product proved to be identical to (—)-O,O-dimethylmagnocurarine iodide (III), obtained by a similar treatment from (—)-magnocurarine, by comparison of the IR spectra and R_f values. Thus, the substituents in colletine are placed in the positions 6, 7 and 4'. The position of the OH group and the two MeO groups was determined through the NMR spectrum.

The NMR spectrum of colletine chloride (Fig. 1) shows two quaternary N-Me groups (singlets at $\delta = 3{\cdot}22$ and 3·48; 3H each), and two MeO groups (singlets at $\delta = 3{\cdot}97$ and 4·00; 3H each). As a MeO group on carbon 7 of tertiary and quaternary benzyltetrahydroisoquinoline bases give signals at $\delta = 3{\cdot}40$–3·60, due to a conformational effect on ring C,[7–11] the only possible structure is II.

This was confirmed by synthesis. Racemic colletine iodide was synthesized following the usual Bischler-Napieralski path,[12] and comparison of the IR and NMR spectra and R_f values showed the identity of the synthetic and natural products.

The absolute configuration of (—)-colletine was established in the following way. Ferrari and Deulofeu[13] demonstrated that (+)-O-methylarmepavine has the L-configuration. Its methiodide (O,O-dimethylmagnocurarine iodide, III), m.p. 136–138°, has an $[\alpha]_D^{20}$ +119° (MeOH). As already mentioned, (—)-colletine iodide can be transformed by methylation into the same product, $[\alpha]_D^{20}$ −106·4° (MeOH), and the same occurs with (—)-magnocurarine: $[\alpha]_D^{20}$ −114·8° (MeOH). Hence, both alkaloids isolated from *Colletia spinosissima* have a configuration opposite to that of L-(+)-O-methylarmepavine, that is, they belong to the D-series; their assymmetric carbon 1 has the R configuration.

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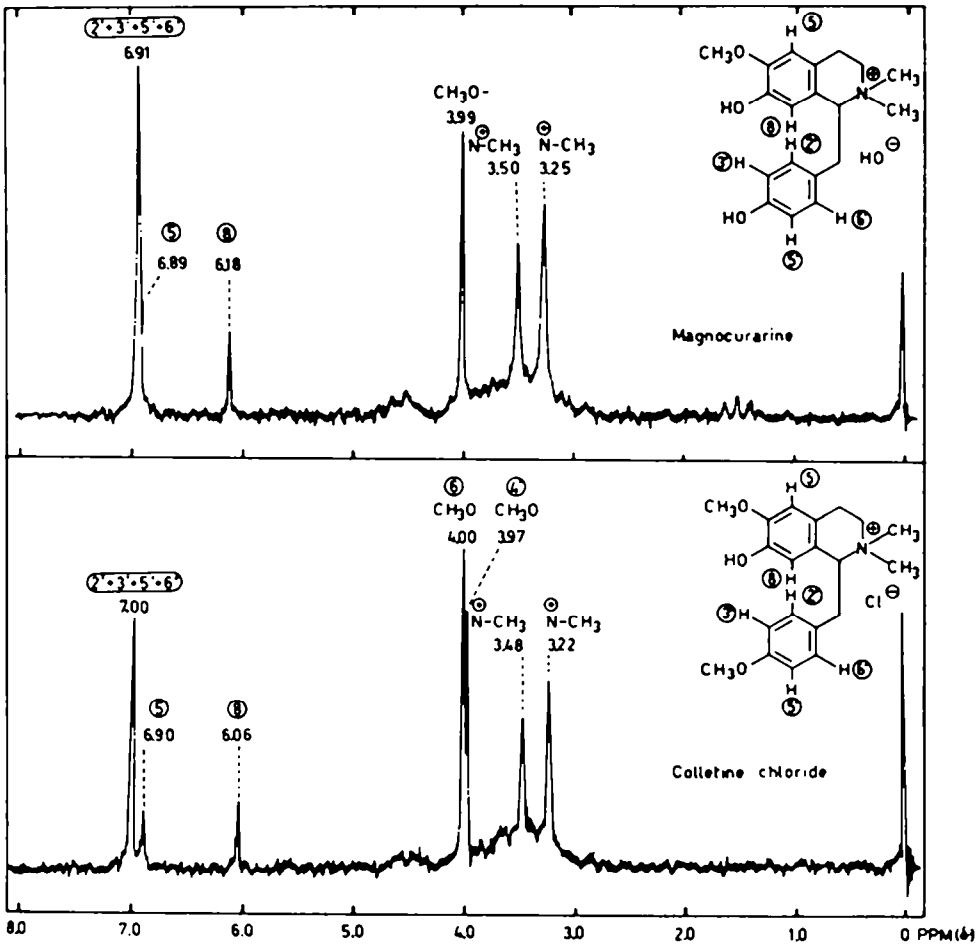

FIG. 1

EXPERIMENTAL

M.ps are uncorrected. Microanalyses were performed by Dr. B. B. de Deferrari and by Dr. A. Bernhardt, Mülheim, Germany. UV spectra: EtOH in a Zeiss RPQ 20C spectrophotometer; IR spectra: a Perkin–Elmer 137B instrument, in nujol mulls; NMR spectra: in trifluoroacetic acid soln in a Varian A-60 spectrometer, with TMS as internal standard. Optical rotations: a Rudolph 70 polarimeter; Ion exchange resins: Amberlite IRA-400, hydroxide form, and Amberlite IRC-50, hydrogen form, unless otherwise stated; Paper chromatography: Whatman 1 paper and the following solvent systems: n-butanol–AcOH–water 10:1:3 (A) and t-butanol–benzene–water 3·1:1:2 (B). TLC on "Avicel" cellulose powder and the solvent system AcOEt–t-butanol–water 4:2:1 (C).

Extraction and separation. The aerial parts of *Colletia spinosissima* were dried at 70° and finely ground. A 3·0 kg sample was then extracted continuously, first with light petroleum ether and then with MeOH. The MeOH extract was evaporated to dryness *in vacuo* on filtercel and the residue taken up in 3·0 l of 0·1N HCl. The resulting suspension was left 3 days at 4° and filtered. The filtrate was extracted with ethyl ether and then brought to pH 7 with anion exchange resin: a ppt formed was discarded. To the filtrate, enough anion exchange resin was added to bring the pH to 10. The alkaline soln was shaked with 150 ml cation exchange resin until the supernatant soln gave a negative Mayer's test. The resin was then packed in a column, throughly washed with water, and the quaternary bases eluted with 1N HCl. The acid eluate was partially neutralized with anion exchange resin and

evaporated to dryness *in vacuo*. The inorganic salts were eliminated by repeated treatment with abs EtOH. The purified residue (11 g) contained two main alkaloids, R_f 0·65 and 0·75 (A). They were separated by a 180 transfers counter-current distribution between 0·1N HCl and n-butanol. Four fractions were obtained: fraction 1 (41·7%, tubes 1–49) contained non-alkaloidical material; fraction 2 (17·3%, tubes 50–70) was formed mainly by the alkaloid R_f 0·65 (A); fraction 3 (14·3%, tubes 71–90) was a mixture of both bases, and fraction 4 (20·7%, tubes 91–180) contained the alkaloid R_f 0·75 (A).

D-(−)-*Magnocurarine* (I)

Fraction 2 of the countercurrent distribution (1·10 g) was dissolved in EtOH (2·0 ml) and chromatographed on alumina (100 g; Woelm, neutral, grade I). Elution with the same solvent gave a fraction (620 mg) containing pure magnocurarine chloride. Part of it (88 mg) was dissolved in water (1·0 ml), and an aqueous soln of sodium picrate added. A gummy ppt was formed which crystallized on cooling (104 mg), and was recrystallized from acetone–water, m.p. 185–185·5°, mixed m.p. with a sample of L-(+)-magnocurarine picrate: 161–181°; and IR spectra identical.

From this picrate (48·5 mg), magnocurarine was obtained by dissolving it in acetone–water 2:3 (10 ml), percolating the soln through a column of anion exchange resin in the HCO_3^- form (2·0 ml), and eluting with the same solvent. The eluate was concentrated *in vacuo* to dryness. The residue (31·5 mg) was crystallized from MeOH–water, m.p. 200–201°, $[\alpha]_D^{20}$ −97·2° (*c*, 0·66, water); UV spectrum: λ_{max} 224 and 283 mμ (log ε_{max} 4·10 and 3·55); IR spectrum: identical to that of an authentic sample; the NMR spectrum is shown in Fig. 1; R_f 0·65 (A), 0·46 (B), 0·15 (C). (Found: C, 68·65; H, 7·70. Calc. for $C_{19}H_{24}NO_4$: C, 68·86; H, 7·60%.)

D-(−)-*Colletine chloride* (II)

Fraction 4 of the countercurrent distribution (600 mg) was dissolved in EtOH (1·0 ml) and chromatographed on alumina (50 g; Woelm, neutral, grade I), eluting with the same solvent. Evaporation of the eluate gave a residue (570 mg) that was recrystallized from AcOEt–EtOH, m.p. 130–132° (Kofler block), $[\alpha]_D^{20}$ −132·8° (*c*, 1·07, EtOH); UV spectrum: λ_{max} 227 and 284 mμ (log ε_{max} 4·22 and 3·69); UV spectrum in alkaline soln: λ_{max} 253 and 303 mμ (log ε_{max} 3·91 and 3·73); the NMR spectrum is shown in Fig. 1; R_f 0·75 (A), 0·59 (B), 0·21 (C). (Found: C, 62·84; H, 7·44; N, 3·65; O, 16·85; Cl, 9·51; MeO, 16·88. $C_{20}H_{26}NO_3Cl·H_2O$ requires: C, 62·55; H, 7·35; N, 3·65; O, 16·67; Cl, 9·23; 2MeO, 16·16%.)

D-(−)-*Colletine iodide* (II)

Colletine chloride (82 mg) was dissolved in water (1·0 ml) and percolated through a column of anion exchanger (2·0 ml). The alkaline eluate was neutralized with aqueous HI and evaporated to dryness. The residue (92 mg) was recrystallized from isopropanol–MeOH, m.p. 169–173°; R_f 0·75 (A), 0·59 (B). (Found: C, 52·72; H, 5·80; N, 3·09; I, 28·04; MeO, 13·42; (N)Me, 8·42. $C_{20}H_{26}NO_3I$ requires: C, 52·75; H, 5·76; N, 3·08; I, 27·87; 2MeO, 13·63; 2(N)Me, 7·84%.)

D-(−)-0,0-*Dimethylmagnocurarine iodide* (III)

(a) *From colletine iodide.* Colletine iodide (22 mg) was dissolved in 0·5N methanolic KOH (0·5 ml) and MeI (0·5 ml) added. The soln was refluxed ½ hr, 0·5N methanolic KOH (0·5 ml) and MeI (0·5 ml) added, and refluxed another ½ hr. The soln was evaporated to dryness and the residue taken up in chf. The chf soln was dried and the solvent removed to give a residue (19·4 mg) which recrystallized from MeOH as needles, m.p. 136·5–138·5°, $[\alpha]_D^{19}$ −106·4° (*c*, 0·98, MeOH); IR spectrum: identical to that of product (b); R_f 0·79 (A), 0·64 (B), 0·41 (C).

(b) *From* D-(−)-*magnocurarine.* To a suspension of D-(−)-magnocurarine (22 mg) in 0·5N methanolic KOH, MeI (0·5 ml) was added. The mixture was refluxed ½ hr, 0·5N methanolic KOH (0·5 ml) and MeI (0·5 ml) was added, and refluxed another ½ hr. The soln was worked up in as in case (a), yielding needles m.p. 136–138°, mixed m.p. with product (a) 136–138°, $[\alpha]_D^{19}$ −114·8° (*c*, 0·76, MeOH); IR spectrum: identical to that of product (a); R_f 0·79 (A), 0·64 (B), 0·41 (C).

(±)-4′-0-*Methylmagnocurarine iodide* (II)

This was synthesized according to Franck and Blaschke,[18] m.p. 188·5–191·5° (lit. 189–190°); IR spectrum (MeCN): identical to that of colletine iodide; NMR spectrum: identical to that of colletine

iodide; R_f 0·75 (A), 0·59 (B). (Found: C, 52·48; H, 5·68; N, 2·96. Calc. for $C_{36}H_{38}NO_4I$: C, 52·75; H, 5·76; N, 3·08%.)

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