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# ISOLATION OF THE ALKALOIDS OF *MONNIERIA TRIFOLIA*

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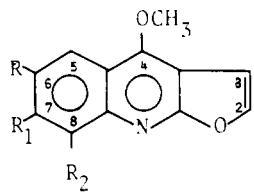
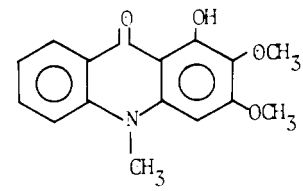
*Monnieria trifolia* L. is an herb that grows throughout northeastern Brazil. The leaves of this plant have been used in popular medicine as a diaphoretic, antipyretic, febrifuge, and an antiinflammatory agent (1). Several alkaloids, typical of the family Rutaceae, have been isolated (2-4) from the leaves of *M. trifolia*. Our interest in the medicinal plants of northeastern Brazil led us to investigate this herb, and we have reported the structures of two furoquinoline alkaloids (5), montrifoline (**1**) and delbine



**1** R=$OCH_2CH(OH)C(OH)(CH_3)_2$; $R_1=OCH_3$; $R_2=H$
**2** R=OH; $R_1=OCH_3$; $R_2=H$
**3** $R=R_1=OCH_3$; $R_2=H$
**4** $R=R_1=H$; $R_2=OCH_3$
**5** $R=H$; $R_1=R_2=OCH_3$
**6** R=H; $R_1=OCH_2CH(OH)C(OH)(CH_3)_2$; $R_2=OCH_3$
**8** $R=OCH_3$; $R_1=OH$; $R_2=H$

(**2**), from the leaves of *M. trifolia*. In this communication, we wish to report the isolation of these alkaloids, along with kokusaginine (**3**), γ-fagarine (**4**), skimmianine (**5**), evoxine (**6**), and arborinine (**7**) from the whole plant. This is the first report of the isolation of kokusaginine and γ-fagarine from this source. The isolation of montrifoline, skimmianine, and arborinine was effected without the use of chromatography.

The spectral properties of montrifoline are very similar to those of kokusaginine, except that the former shows the presence of an isopentoxy group in place of a $OCH_3$ group. Upon KOH fusion, montrifoline furnished delbine with the loss of a $C_5$-unit ($C_5H_{10}O_2$). That this $C_5$-unit is a $(CH_3)_2C(OH)CH(OH)CH_2$ side-chain, is indicated by the pmr, ms (see below), and by biogenetic considerations. Delbine gives kokusaginine upon methylation, and as delbine has a $OCH_3$ group at C-4 (3H singlet at 4.37 ppm), it must be either 6-hydroxy-7-methoxydictamnine (**2**) or 7-hydroxy-6-methoxydictamnine (**8**). However, structure **8** represents heliparvifoline, mp 245-247°, an alkaloid isolated from *Helietta parvifolia* (6) and proved to be different from delbine by direct comparison with an authentic sample. Therefore, structure **2** should represent delbine, and, consequently, structure **1** should be assigned to montrifoline (5).



**7**

## EXPERIMENTAL

MATERIALS.—The plant material was collected from an area within 150 km of João Pessoa in the northeastern state of Paraiba, and the herbarium specimen is kept in the LPX Herbarium of the Universidade Federal da Paraiba. The mps were taken on a Kofler hot stage and are uncorrected. The uv spectra were determined in a Carl Zeiss Jena VSU 2-P spectrophotometer, and the ir spectra were obtained in a Perkin-Elmer 467 grating spectrophotometer. A 60 MHz Varian EM 360A apparatus was used for the pmr spectra, and the mass spectra were determined in a Hewlett-Packard 5930 quadrupole mass spectrometer. Extracts of $CHCl_3$ were dried over anhydrous $Na_2SO_4$. Sodium carbonate was used for basification of acid solutions. Silica gel (E. Merck, No. 7734) was used for analytical tlc plates and silica gel of E. Merck (60 $PF_{254}$) was used for preparative tlc.

EXTRACTION AND PURIFICATION OF THE ALKALOIDS.—*M. trifolia* (whole plant), dried and ground, (13.0 kg) was extracted with hexane for 8 h followed by 95% EtOH for 14 h in a Soxhlet apparatus. The hexane extract, after standing in cold overnight, gave a dark greenish yellow precipitate which was collected by filtration (fraction A). The filtrate was evaporated to dryness (fraction B). The ethanolic erxtract, likewise, was evaporated to dryness (C, 942.0 g).

Fraction B was dissolved in $CHCl_3$ (1.0 liter) and was thoroughly extracted with 1 *N* $H_2SO_4$ until the last extract was free from alkaloids. The $CHCl_3$ phase was washed with $H_2O$, dried, and evaporated to give a non-alkaloidal fraction, which was left aside for future investigation. The combined acid-aqueous layer was cooled, basified to pH ~4, and extracted thoroughly with $CHCl_3$ (5×400 ml). The organic layer, after usual work-up, gave a brown residue ($B_1$, 18.0 g). The aqueous layer was discarded.

A part of fraction C (470.0 g) was then treated with 1 *N* $H_2SO_4$ (1.2 liter), stirred well, and filtered. The residue was left aside for future investigation. The acid-aqueous filtrate was cooled, basified to pH ~4, and extracted thoroughly with $CDCl_3$ (4×400 ml). The organic phase, after usual work-up, gave a yellowish brown solid ($C_1$, 17.4 g). The acid aqueous layer was found to be practically free from alkaloids and was discarded.

Fraction $B_1$ (18.0 g) was triturated with absolute EtOH when a crystalline solid separated, which was collected by filtration ($B_2$, 6.2 g). The filtrate ($B_3$) was left aside for further treatment.

ISOLATION OF ARBORININE (**7**).—Arborinine was obtained from Fraction A by treatment with activated charcoal in ethanolic solution followed by crystallization from $Me_2CO$-hexane in fine yellow needles, mp 178-179°, which showed spectral properties (uv, ir, pmr, and ms) identical to those published for arborinine (7).

ISOLATION OF SKIMMIANINE AND KOKUSAGININE.—Fraction $B_2$ was recrystallized several times to give a light cream-colored crystalline solid (4.1 g), mp 176°. The solid showed spectral characteristics (uv, ir, pmr, and ms) practically identical to those published for skimmianine (8-10). The mother liquor showed two spots in an analytical tlc plate developed with $CHCl_3$-$CH_3OH$ (98:2). Therefore, this mixture was subjected to preparative tlc plates and developed with the same solvent system. The two principal bands were cut, and the bases were extracted with $CHCl_3$-$CH_3OH$ (9:1). The residue from the lower band was found to be identical to skimmianine. The residue from the upper band, after recrystallization from $C_6H_6$, gave very light cream-colored crystals, mp 171°. The physical data (uv, ir, pmr, ms) were almost identical to those published for kokusaginine (9-11).

ISOLATION OF MONTRIFOLINE (**1**).—Fractions $C_1$ and $B_3$ were combined to give a crude mixture (29.0 g) that was dissolved in $CHCl_3$ and extracted with dilute $H_2SO_4$ (pH 4; 2×140 ml). The acid extract at pH 4 was basified to pH 9, and the precipitated bases were extracted in the usual way to give a crude alkaloid fraction (1.5 g), which was left aside for future investigation. The $CHCl_3$ solution was then extracted with 1 *N* $H_2SO_4$ (150 ml), and the acid extract was basified. Extraction of the precipitated bases afforded a fraction (D, 17.0 g), which was again dissolved in $CHCl_3$. The material separated was collected by filtration (0.35 g). The filtrate ($D_2$) was extracted with dilute $H_2SO_4$ (pH 1.5; 3×150 ml). The combined acid-aqueous layer was basified, and the precipitated alkaloids were extracted in the usual way to give a yellowish brown solid ($D_3$; 7.02 g). The $CHCl_3$ solution was left aside for future investigation. Fraction $D_3$ ws taken up in hot $CHCl_3$ when a white solid separated, which was collected by filtration ($D_4$; 2.3 g). The filtrate was evaporated to give a residue ($D_5$; 4.9 g), which was left aside for chromatography. Fraction $D_1$ and $D_4$, which showed similar characteristics in tlc, were combined and recrystallized several times from $CHCl_3$-$CH_3OH$ to give white crystals (0.6 g) of montrifoline, $C_{18}H_{21}NO_6$ ($M^+$ 347), mp 191-193°.

*Anal.* calcd. for $C_{18}H_{21}NO_6$: C, 62.24; H, 6.05; N, 4.03. Found: C, 62.43; H, 5.91; N, 4.01; ir (KBr) 3350, 1630, 1509, 1268, 1210, 845, 820 $cm^{-1}$; pmr (DMSO-$d_6$) 1.26 (6H, *s*, broadened at the top), 3.96 (3H, *s*), 4.46 (3H, *s*), 7.28 (1H, d, $J$=3 Hz), 7.28 (1H, *s*), 7.53 (1H, *s*), 7.82 (1H, *d*, $J$=3 Hz), 4.00 (1H, *m*), and 4.40 (2H, *m*), ppm; ms *m/z* 347 ($M^+$), 332, 288, 258, 245 (base peak); uv λ max (log ε), 244 (4.68), 252 (4.73), 309 (3.99), 321 (3.99), 333 (3.82) nm.

Fraction $D_5$ (4.9 g) was chromatographed over a column of silica gel (140 g), and the column was eluted with $CHCl_3$ followed by $CHCl_3$-MeOH mixtures of increasing polarity. Fractions of 250 ml were collected, and the fractions with similar composition were added together. Thus, three main fractions were collected; fractions 4-5 ($D_6$), 13-50 ($D_7$), and 146-154 ($D_8$).

ISOLATION OF γ-FAGARINE.—Fraction $D_6$ was crystallized several times from $C_6H_6$-hexane (0.05 g), mp 140-142°. The identity of this compound with γ-fagarine was confirmed by direct comparison (mmp, ir, tlc) with an authentic sample.

ISOLATION OF DELBINE (**2**).—Fraction $D_7$, which contained mainly skimianine and another

compound (tlc), was crystallized two times, when most of the skimmianine was removed. The residue was subjected to preparative tlc; the plates were developed with $CHCl_3$-MeOH (98:2), and two bands were collected. The lower band, which showed a pink fluorescence under uv light, after usual work-up, gave a white solid that was crystallized from MeOH, mp 229-231°.

*Anal.* calcd. for $C_{13}H_{11}NO_4$: C, 63.37; H, 4.49; N, 5.71. Found: C, 63.21; H, 4.53; N, 5.57; uv λ max (log ε) 241 (4.17), 250 (4.19), 310 (3.51), 326 (3.88); ir (KBr) 3400, 3100, 1623, 1585, 1265, 1210, 1088, 865, 850 $cm^{-1}$; pmr (DMSO-$d_6$) 3.95 (3H, *s*), 4.37 (3H, *s*), 7.29 (1H, *s*), 7.32 (1H, *d*, *J*=3 Hz), 7.48 (1H, *s*), 7.90 (1H, *d*, *J*=3 Hz), 9.60 (1H, *s*, broad) ppm; ms *m/z* 245 ($M^+$, base peak), 230, 202.

ISOLATION OF EVOXINE (**6**).—The chromatographic fraction $D_8$, after several crystallizations from MeOH, gave crystals, mp 154-155°. The identity of this alkaloid was confirmed by direct comparison (mmp, tlc, ir) with an authentic sample.

DELBINE (**2**) FROM MONTRIFOLINE (**1**).—Montrifoline (0.2 g) was heated in a melt of KOH for 2 min at 160°. The melt was then treated in the usual way, and the product was purified by preparative tlc using $CHCl_3$-MeOH (98:2) as developer. The upper band with pink fluorescence was cut, and the product, after usual work-up, was found to be identical to natural delbine (mp, mmp, ir, pmr).

KOKUSAGININE (**3**) FROM DELBINE (**2**).—Delbine (0.05 g) was methylated with $CH_2N_2$ in the usual manner. The product, after usual work-up, was found to be identical to natural kokusaginine in all respects (mp, mmp, ir, pmr).

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