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ISOLATION OF DIHYDROCUSCOHYGRINE FROM PERUVIAN COCA LEAVES

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Key Word Index—*Erythroxylon coca*; Erythroxylaceae; coca leaves; alkaloids; cuscohygrine; (−)-dihydrocuscohygrine; identification.

Abstract—An investigation of the alkaloidal fraction of *Erythroxylon coca* leaves, collected in Peru, has resulted in the isolation and characterization of cuscohygrine and (−)-dihydrocuscohygrine. The structure of (−)-dihydrocuscohygrine was deduced by spectral and chemical means.

INTRODUCTION

In previous communications we have reported extraction procedures and the determination of cocaine [1] and *cis*- and *trans*-cinnamoylcocaine [2] in Peruvian coca. In our latter communication [2] we also compared the GC analysis of extracts of coca using a nitrogen and FID detector and confirmed the analyses by GC/MS. In that report four unidentified nitrogenous compounds appearing before cocaine were detected. GC/MS analysis of these components showed that these peaks are probably related to ecgonine. They showed M^+ at *m/z* 181, 181, 199, and 213, respectively, in order of increasing retention times.

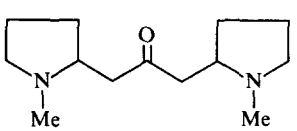
In our efforts to identify these components we isolated cuscohygrine and a new compound which we identified as (−)-dihydrocuscohygrine. Cuscohygrine is one of the major alkaloids [3] reported in the leaves of Bolivian and Peruvian *Erythroxylon coca*. This work describes the isolation and characterization of these two alkaloids from Peruvian coca.

RESULTS AND DISCUSSION

The phytochemical examination of the alkaloids of *E. coca* and other species of the genus *Erythroxylon* has been the subject of previous reports [3–10]. The most important commercial constituent of coca leaves is the alkaloid cocaine. GC/MS fragmentographic analysis of extracts from the leaves of 13 different species of *Erythroxylon* showed that only two cultivated species contained cocaine, namely *E. coca* and *E. novagranatense* [11]. In our laboratory, a simple GC procedure was developed for the determination of cocaine and the *cis*- and *trans*-isomers of cinnamoylcocaine in extracts from coca leaves [2]. The presence of peaks of other nitrogenous compounds in the chromatograms of ethanolic extracts of coca leaves collected in Peru prompted our investigation. Repeated chromatography of the alkaloidal fraction of the ethanol extract of the leaves collected in the Tingo-Maria area of Peru, resulted in the isolation of two related alkaloids, namely cuscohygrine (**1**) and (−)-dihydrocuscohygrine (**2**).

Liebermann and Cybulski [12] were first to isolate and study the alkaloid cuscohygrine from *E. coca*. Hegnauer and Finkenscher [3] reported cuscohygrine as one of the major alkaloids in Bolivian and Peruvian coca leaves. Hess and Fink [13] found that natural cuscohygrine was optically inactive and according to Hess and Anselm [14] could not be resolved into active components. In an attempt by Hess and Bappert [15] to prove the structure of natural cuscohygrine, the latter was reduced with Na/Hg or Na/EtOH to give two alcohols. This indicated that natural cuscohygrine had a *meso* configuration. However, this assumption could not explain the Hess and Fink [13] report of the two isomeric hydrazones. The suggestion of Sohl and Shriner [16] that the natural material is a mixture of *meso* and racemic forms would account for the two hydrazones, but would lead to three rather than two alcohols [19]. The synthesis of cuscohygrine has been the subject of several reports [17–21] and its stereochemistry was studied by Galinovsky and Züber [22].

In our laboratory, cuscohygrine (**1**) was isolated as a yellow, optically inactive oil. The presence of a carbonyl



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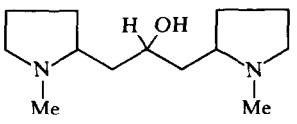

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absorption at 1718 cm^{-1} indicated a ketone with a MW of 224. In addition, the ^1H NMR spectrum showed a peak at δ 2.35 ($N-Me$) which integrated for six protons.

Dihydrocuscohygrine (**2**), on the other hand, was isolated as an optically active oil ($[\alpha]_D$ − 68°). Mass spectral data showed a M^+ at m/z 226 for $C_{13}H_{26}N_2O$. The IR spectrum showed peaks for hydrogen-bonded OH at 3150 and other peaks at 1460, 1370, 1105, 1060 cm^{-1}. The ^1H NMR spectrum showed signals at δ 1.5–2.16 (12 H, *m*) and δ 2.45 (*s*) integrated for six protons (N − Me) and others at δ 3.2 (6 H, *m*), δ 4.1 (1 H, *m*), and δ 5.9 (1 H, *br. s*). These data suggested that the compound could be the dihydro derivative of cuscohygrine. This was confirmed by sodium borohydride reduction of cusohygrine which resulted in three compounds: R_f 0.27, 0.47 and 0.53 using cyclohexane/chloroform/diethylamine (5:4:1) as a solvent system. Since all three compounds isolated from the reduction product of natural cuscohygrine were optically inactive, it follows that the starting cuscohygrine was a mixture of the *meso* and *dl*-forms. One compound (**4B**), isolated from the reduction product of cuscohygrine, had an R_f value of 0.47 which corresponded to that of natural dihydrocuscohygrine. It showed two signals in the ^1H NMR for the N-methyls at δ 2.41 and δ 2.46. These data suggested that the synthetic dihydrocuscohygrine (**4B**) represents the reduction product of the *dl*-form of cuscohygrine.

Since natural dihydrocuscohygrine had the same R_f value as **4B**, and its ^1H NMR spectrum showed one signal for N-methyls at δ 2.45, but was optically active, then we conclude that natural dihydrocuscohygrine represents the reduction product of the **1**-form of cuscohygrine, namely (−)-1,3-bis(1′-methyl-2′-pyrrolidyl)-2-propanol.

EXPERIMENTAL

IR spectra were recorded in $CHCl_3$. ^1H NMR spectra were measured at 60 MHz in $CDCl_3$ with TMS as int. standard. Optical rotations were determined for *ca* 1% solns in Me_2CO at 24°. MS were determined at 70 eV. GC analysis was carried out according to published procedures [1, 2].

Plant material was collected from the Tingo-Maria area of Peru and voucher specimens were deposited in the Herbarium, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi.

Extraction and isolation. Erythroxylon coca Lam. leaves (4.7 kg) were extracted with 95% EtOH for 72 hr at room temp. (×6). The extract after evaporation to dryness (375 g) was partitioned between $CHCl_3$ and 2% HCl. The aq. acidic extract was made alkaline (pH 10) using NH_4OH and extracted with $CHCl_3$ (34 g, 1A). Chromatography of 1A (10 g) on a Si gel G column (300 g, 5 × 45 cm) packed in cyclohexane–$CHCl_3$–Et_2NH (8:1:1, system A) yielded 10 fractions (2A–2J).

Isolation of cuscohygrine (**1**). Fraction 2G (1.4 g) was rechromatographed on a Si gel G column (45 g, 3 × 33 cm) using cyclohexane–$CHCl_3$/Et_2NH (5:4:1, system B) and 5 fractions were collected (3A–3E). Fractions 3C and 3D (510 mg) were combined. Part of this residue was further purified using preparative chromatography on precoated Si gel G (0.5 mm thickness) and the same developing system as described above to yield a light yellow oil (70 mg) identified as **1**: $[\alpha]_D$ 0; RR_t 0.46; R_f 0.34 (system B); IR ν_{max} cm^{-1}: 2973, 2960, 2810, 1718, 1465 and 1384; MS m/z (rel. int.) 224 (M^+ 1), 209 (2), 181 (1), 154 (1), 142 (36), 124 (15), 108 (4), 98 (29) and 84 (100); ^1H NMR ($CDCl_3$): δ 1.4–2.2 (12 H, *m*), 2.35 (6 H, *s*, N–Me), 2.36–2.73 (4 H, *m*), 2.93–3.2 (2 H, *m*).

Isolation of (−)-dihydrocuscohygrine (**2**). Fraction 2F (1.6 g) was further fractionated on a Si gel G column (35 g, 2 × 35 cm) using system B and 6 fractions were collected (3A–3F). Prep. TLC of fraction 3C, under the same conditions described above, afforded a yellow coloured oil (33.5 mg) identified as (**2**): $[\alpha]_D$ −68° (*c* 2.5, Me_2CO), RR_t 0.49: R_f 0.47 (system B); IR ν_{max} cm^{-1}: 3150, 2970, 2850, 2800, 1460, 1370, 1105 and 1060; MS m/z (rel. int.): 226 (M^+22), 211 (24), 208 (6.5), 142 (17), 128 (48), 110 (15), 98 (43), 84 (100): ^1H NMR ($CDCl_3$): δ 1.5–2.16 (12 H, *m*), 2.45 (6 H, *s*, N–Me, 3.2 (6 H, *m*), 4.1 (1 H, *m*), δ 5.9 (1 H, *br.* exchangeable with D_2O).

Reduction of cuscohygrine to dihydrocuscohygrine. To 40 mg of **1** in EtOH (3 ml) was added excess $NaHB_4$ and the reaction mixture stirred for 1 hr at 0°. Excess $NaHB_4$ was destroyed by the addition of H_2O and 1 drop of NH_4OH and the reaction product extracted with $CHCl_3$ (3 ×15 ml). The combined $CHCl_3$ extracts were dried (Na_2SO_4) and evaporated to dryness. TLC using system B showed the presence of 3 spots with R_f values of 0.27, 0.47, and 0.53. Each component was separated using prep. TLC and system B developing solvent. The 3 compounds were obtained as pale yellow oils and named **4A**, **4B**, and **4C** (R_f and wts): 0.27 (14 mg), 0.47 (9 mg), and 0.53 (7 mg), respectively.

4A. $[\alpha]_D$ = 0; IR ν_{max} cm^{-1}: 3200, 2980, 1470, 1139, 1109; MS m/z (rel. int.): 226 (M^+); 211, 208, 142, 128, 84 (base peak); ^1H NMR ($CDCl_3$): δ 1.20–2.03 (12 H, *m*), 2.45 (6 H, *s*, N–Me), 3.0–3.16 (6 H, *m*), 3.6 (1 H, *m*), 4.6 (*br. s*).

4B. $[\alpha]_D$ 0; IR ν_{max} cm^{-1}: 3200, 2975, 2805, 2805, 2862, 1465, 1138, 1110; MS m/z (rel. int.) 226 (M^+), 211, 208, 142, 128, 84 (base peak); ^1H NMR ($CDCl_3$): δ 1.16–2.13 (12 H, *m*), 2.41 (3 H, *s*, N–Me), 2.46 (3 H, *s*, N–Me), 3.0–3.4 (6 H, *m*), 4.06 (1 H, *m*), 5.83 (1 H, *br. s*).

4C. $[\alpha]_D$ 0; IR ν_{max} cm^{-1}: 3100, 2970, 2852, 2905, 1584, 1485, 1460, 1365, 1134, 1110; MS m/z (rel. int.): 226 (M^+), 211, 208, 142, 128, 84 (base peak); ^1H NMR ($CDCl_3$): δ 1.56–2.33 (12 H, *m*), 2.43 (6 H, *s*, N–Me), 3–3.33 (6 H, *m*), 4.16 (1 H, *m*), 4.65 (*br. s*).

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