Journal of Natural Products
Vol. 53, No. 3, pp. 666-668, May-Jun 1990

TWO NEW BENZYLTETRAHYDROISOQUINOLINE ALKALOIDS FROM *ROEMERIA REFRACTA*

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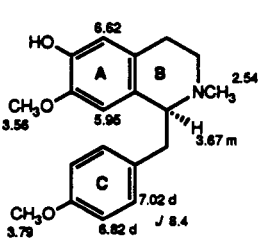
ABSTRACT.—*Roemeria refracta* of Turkish origin produces the new benzyltetrahydroisoquinoline alkaloids (+)-roefractine [**1**] and (+)-pseudolaudanine [**2**], together with the known (+)-roemecarine [**3**] and (−)-armepavine.

As part of an ongoing study of the alkaloids of Turkish *Roemeria* (Papaveraceae) species, we had occasion to investigate the content of a fraction of *Roemeria refracta* DC. obtained by elution of the alkaloids through a Si gel column using 5% MeOH in $CHCl_3$. This fraction was further purified by tlc to yield two new benzyltetrahydroisoquinoline (BTHI) alkaloids, (+)-roefractine [**1**] and (+)-pseudolaudanine [**2**].

The uv spectrum of (+)-roefractine [**1**], $C_{19}H_{23}NO_3$, displayed a maximum at 285 nm, suggesting that the molecule incorporated a tetrahydroisoquinoline system. Furthermore, a bathochromic shift upon addition of base made it probable that a phenolic function was present. The ^{1}H-nmr spectrum (360 MHz, $CDCl_3$), outlined around structure **1**, displayed an AA′BB′ system at δ 6.82 and 7.02 reminiscent of a coclaurine-type base. Three-proton singlets at δ 3.56 and 3.79 represented two methoxyl groups. It is well known that 7-MeO in coclaurine-type alkaloids resonates at δ 3.51–3.55 (1). Presently, the upfield shift at δ 3.56 was in line with a methoxyl at C-7. In general, in 6-hydroxy-7-methoxy BTHIs, H-8 resonates between δ 5.8 and 6.0, whereas the range is from δ 6.3 to 6.4 when the positions of these substituents are reversed. The chemical shift of the H-8 singlet of **1** fell at δ 5.95, supporting the assumption that we were indeed dealing with a 6-hydroxy-7-methoxy substitution pattern.

In the eims, the abundance of the molecular ion *m/z* 313 was less than 1%, while the base peak, *m/z* 192, corresponded to the *N*-methylisoquinolinium cation with one methoxyl and one hydroxyl substituent. An additional prominent peak, *m/z* 177 (24%), was formed by loss of a methyl radical from the base ion. A cims in CH_4, however, clearly showed *m/z* 313 as the molecular ion.

In order to ascertain the positions of the substituents, a partial ^{1}H-nmr nOe experiment was carried out. Irradiation of the δ 5.95 and then the δ 3.56 peaks showed strong reversible nOe's. On the other hand, irradiation of the other aromatic proton singlet (δ 6.62) effected only enhancement of H-4 (δ 2.70–2.88). Irradiation of the other



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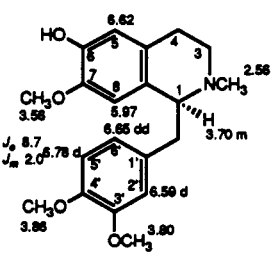

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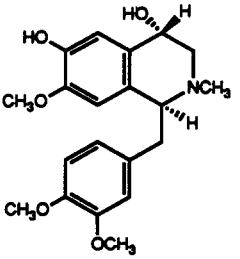

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methoxyl singlet (δ 3.79) enhanced the aromatic doublet at δ 6.82, thus confirming the position of this methoxyl at C-4′.

The cd curve of (+)-roefractine [**1**] displayed prominent maxima at 235 and 290 nm and was almost identical with that reported for (+)-laudanosine possessing the C-1 *S* configuration (2). It follows that (+)-roefractine [**1**] also incorporates the *S* configuration at its chiral center.

Final proof of structure was obtained through CH_2N_2 *O*-methylation of **1**, which gave rise to (+)-*O*-methylarmepavine, identical (nmr, mass, cd, tlc) with an authentic sample in our possession, as well as with literature values (3).

(+)-Roefractine [**1**] had been previously obtained as a product of the lithium in liquid NH_3 cleavage of the bisbenzylisoquinoline (+)-isotetrandrine (1,4). The present study is the first report of the natural occurrence of this BTHI.

The ^{1}H-nmr spectrum of our second alkaloid, (+)-pseudolaudanine [**2**], $C_{20}H_{25}NO_4$, showed the chemical shifts of the aromatic singlets (δ 5.97 and 6.62) to be very close to those of (+)-roefractine [**1**], suggesting the identical substitution pattern on ring A. However, the remaining three aromatic protons displayed an ABX pattern, pointing to C-3′, -4′ substitution on ring C. Three methoxyl resonances were clearly in evidence at δ 3.56, 3.80, and 3.86, the former attributable to 7-MeO (1).

Once again, a partial nmr nOe experiment was performed to define the substitution on ring A and to assign the chemical shifts of the methoxyl groups. The H-8 singlet at δ 5.97 and the upfield 7-MeO singlet at 3.56 displayed reversible nOe's. Irradiation of H-8 also enhanced the H-1 signal (δ 3.70). Saturation of the second aromatic singlet (δ 6.62) only caused enhancement of the H-4 benzylic protons (δ 2.70–2.88). Finally, irradiation of the 3′-MeO signal (δ 3.80) affected the meta-coupled H-2′ doublet (δ 6.59), whereas irradiation of 4′-MeO (δ 3.86) resulted in enhancement of the ortho-coupled H-5′ (δ 6.78).

The eims of **2** furnished only a very small molecular ion *m/z* 343 (0.1%). The base peak, *m/z* 192, corresponded to the same isoquinolinium fragment observed in the mass spectrum of (+)-roefractine [**1**]. A cims in isobutane showed *m/z* 343 as the molecular ion, thus confirming the proposed structure **2**.

The uv maximum at 282 nm as well as the distinct bathochromic shift in alkali gave added support to the structural assignment. The cd spectrum of **2** was generally similar to that of **1**, with maxima at 213, 238, and 288 nm. It follows, therefore, that alkaloid **2** also has the C-1 *S* configuration.

O-Methylation of (+)-pseudolaudanine [**2**] gave (+)-laudanosine, identified by its spectra (nmr, mass, cd) as well as by comparison with an authentic sample.

Synthetic pseudolaudanine had been obtained by Burger (5) more than sixty years ago as one of the four partial *O*-demethylation products of racemic laudanosine, but the natural occurrence of (+)-pseudolaudanine [**2**] is reported for the first time in the present study.

It should also be mentioned that a quaternary *N*-metho salt of pseudolaudanine had been isolated as its styphnate from *Fagara mayu* (Rutaceae) under the name *N*-methylpseudolaudanine, but no indication of its optical activity was given (6). At a later date, the dextrorotatory methiodide salt of pseudolaudanine was obtained from *Papaver pseudo-orientale* (Papaveraceae) and was assigned the name (+)-pseudorine (7).

The alkaloidal fraction presently under investigation contained, in addition to the above two new alkaloids, two known BTHI bases, namely (+)-roemecarine [**3**] and (−)-armepavine. The former is a 4-hydroxylated BTHI originally isolated from *Roemeria carica* (8,9). Its reisolation from *R. refracta* suggests that this alkaloid may be a common element of the alkaloidal profile of this genus.

Perusal of the order of substitution in structures **1–3** also suggests that the 6-hydroxy-

7-methoxy pattern for the *Roemeria* BTHI may be fairly common. Additionally, the trioxygenated BTHI of *Roemeria* species may belong to either the *S* or the *R* configurations, as exemplified by (−)-armepavine and by (+)-roefractine [**1**].

EXPERIMENTAL

PLANT MATERIAL.—*R. refracta* was collected near Bayburt in Gümüşhane Province in eastern Turkey on June 21, 1988. The plant was identified by T.G., and a voucher specimen, No. 1092, was deposited in the Herbarium of Pharmacognosy, Faculty of Pharmacy, Ege University.

EXTRACTION AND FRACTIONATION OF ALKALOIDS.—The dried and ground plant material (16.7 kg) was extracted with EtOH (360 liters) at room temperature. The residue after solvent evaporation (1 kg) was taken up in 5% HCl and filtered. The acidic filtrate was basified with NH_4OH and extracted with $CHCl_3$. Evaporation of the solvent yielded the crude alkaloids (25 g). Preliminary cc was on Si gel (70–230 mesh, Merck) using $CHCl_3$ gradually enriched with MeOH. The fraction (886 mg) eluted with 5% MeOH in $CHCl_3$ was further separated on a Si gel 60H column using $CHCl_3$-Me_2CO-MeOH-NH_4OH (80:14:6:0.5). Final purification was by tlc on Si gel glass plates. The following alkaloids were obtained: (+)-roefractine [**1**] (28 mg), (+)-pseudolaudanine [**2**] (32 mg), (+)-roemecarine [**3**] (51 mg), and (−)-armepavine (6 mg). All nmr spectra are in $CDCl_3$ and were obtained at 360 MHz.

(+)-ROEFRACTINE [**1**].—Amorphous, $[\alpha]_D$ +73° (c = 0.14, MeOH); uv λ max (MeOH) 226, 285, 292 sh nm (log ε 4.18, 3.64, 3.48); uv λ max (MeOH + OH^-) 245 sh, 279 sh, 287, 296, 308 sh nm (log ε 3.85, 3.53, 3.59, 3.58, 3.47); eims *m/z* (%) 193 (13), 192 (100), 177 (24), 163 (2), 149 (4), 148 (6), 132 (2), 121 (8); cims (CH_4) *m/z* $[M]^+$ 313; cd (MeOH) Δε (nm) 0 (296), +1.49 (290), 0 (286), −3.23 (272), −2.61 (263), −3.52 (250), 0 (242), +8.69 (235), +0.75 (220), positive tail beyond 220 nm. Relevant nmr nOe's are H-8 to 7-MeO (20%), H-8 to H-1 (13%), H-8 to H-2′ (5%), 7-MeO to H-8 (24%), H-5 to H-4 (12%), 4′-MeO to H-3′ (38%).

O-METHYLATION OF (+)-ROEFRACTINE.—Alkaloid **1** (5 mg) in MeOH (1 ml) was treated with excess ethereal CH_2N_2 and kept at near 0° overnight. Workup afforded (+)-*O*-methylarmepavine (3.5 mg).

(+)-PSEUDOLAUDANINE [**2**].—Amorphous, $[\alpha]_D$ +52° (c = 0.07, MeOH); uv λ max (MeOH) 226 sh, 282 nm (log ε 4.08, 3.72); uv λ max (MeOH + OH^-) 233 sh, 286, 301 sh nm (log ε 4.06, 3.66, 3.54); eims *m/z* (%) 193 (13), 192 (100), 190 (4), 178 (3), 177 (19), 151 (5), 149 (4), 148 (5); cims (isobutane) *m/z* $[M]^+$ 343; cd (MeOH) Δε (nm) 0 (297), +1.36 (288), 0 (279), −1.12 (267 sh), −1.76 (252), 0 (247), +3.68 (238), 0 (229), −0.76 (227), 0 (226), positive tail beyond 226 nm. Relevant nmr nOe's are H-8 to 7-MeO (27%), H-8 to H-1 (13%), H-8 to H-6′ (5%), 7-MeO to H-8 (23%), H-5 to H-4 (24%), 4′-MeO to H-5′ (32%), 3′-MeO to H-2′ (29%).

O-METHYLATION OF (+)-PSEUDOLAUDANINE.—Alkaloid **2** (7 mg) in MeOH (1 ml) was treated with excess ethereal CH_2N_2 at near 0° overnight. Workup yielded (+)-laudanosine (5 mg).

ACKNOWLEDGMENTS

This research was supported by NSF grant INT-8814406. B.G. was the recipient of International Foundation for Science grant F/990-1.

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Received 29 November 1989