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SOME UNUSUAL PAVINE AND ISOPAVINE ALKALOIDS FROM *ROEMERIA REFRACTA*

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ABSTRACT.—*Roemeria refracta* of Turkish origin has yielded (−)-eschscholtzinone [**5**], the first ketonic pavinoid, and (−)-norreframidine [**2**], the only known naturally occurring *N*-norisopavine. The new isopavine (−)-refractamine [**3**], structurally isomeric with the known (−)-reframoline [**4**], was also obtained. High resolution ^{1}H-nmr data are provided for the first time for the isopavines.

Prior to the initiation of the present work, certain structural features appeared to be common to all pavine and isopavine alkaloids. First, no naturally occurring *N*-norpavines or *N*-norisopavines were known. Second, none of the known pavines or isopavines possessed ketonic functions. These trends were somewhat unusual, because *N*-nor bases, as well as ketonic functions, commonly occur among several other types of isoquinoline alkaloids (1).

The genus *Roemeria*, belonging to the family Papaveraceae, counts seven species spread out from the Mediterranean to Central Asia. We have previously reported on *Roemeria hybrida* (2), which is rich in proaporphines; we now describe some of the alkaloids of *Roemeria refracta* DC. *R. refracta*, previously studied by Czech (3) and Russian groups (4–8), had supplied four isopavines, namely (−)-reframidine [**1**], (−)-reframine, (−)-reframoline [**4**], and (−)-remrefine, together with a variety of proaporphines, aporphines, and other isoquinoline-type bases. Of the aforementioned four isopavines, we were able to reisolate the first three, (−)-reframidine [**1**], (−)-reframine, and (−)-reframoline [**4**].

Of greater interest, however, is the fact that we also obtained two new isopavines and one pavine. The first isopavine, (−)-norreframidine [**2**], is the only *N*-nor isopavine base recognized to occur naturally; while the second isopavine, (−)-refractamine [**3**], is structurally isomeric with the known (−)-reframoline [**4**]. Our new pavine, (−)-eschscholtzinone [**5**], is the first known ketonic pavinoid.

(−)-Norreframidine [**2**], $C_{18}H_{15}NO_4$, λ max (MeOH) 217, 233 sh, 246 sh, 296 nm (log ε 4.09, 3.81, 3.62, 3.92), had a mass spectrum with molecular ion *m/z* 309 and base peak *m/z* 280 due to loss of CH_2NH from the molecular ion (9).

The 360-MHz nmr spectrum in $CDCl_3$ has been summarized around structure **2**. Through decoupling experiments, it was possible to ascribe specific chemical shifts to the aliphatic hydrogens. This represents the first time that such detailed assignments have been made for an isopavine. Ring B is in a near boat conformation. The H_a/H_c dihedral angle is close to 76°, resulting in no noticeable coupling between the hydrogens in question. On the other hand, the H_a/H_b angle is about 32°, leading to $J_{a,b} = 4.6$ Hz (10). Additionally, H_e and H_f are split almost to the same extent by H_d, with $J_{d,e} = 3.3$ Hz and $J_{d,f} = 3.5$ Hz, for dihedral angles H_d/H_e and H_d/H_f of approximately 54°. Final proof of structure was provided by *N*-methylation with formaldehyde and $NaBH_4$, which supplied (−)-reframidine [**1**].

The nmr spectrum of (−)-reframidine is given around structure **1**. Chemical shift assignments for the aliphatic protons were confirmed by decoupling experiments, as well as by nOe studies. The conformation of the molecule varies slightly from that of its *N*-nor analogue as indicated by the unequal splittings between H_d and H_f on the one

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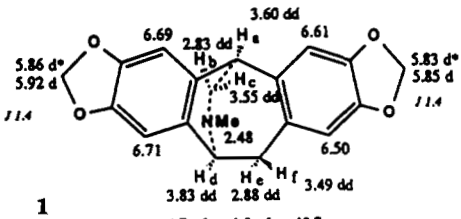
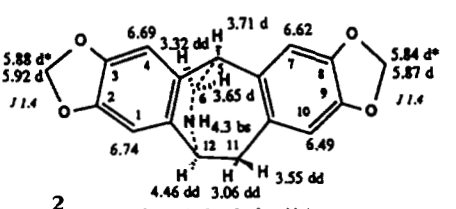


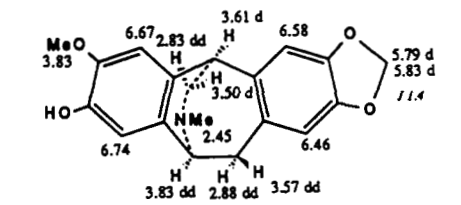
1

$J_{a,b}$ 4.7; $J_{a,c}$ 1.5; $J_{b,c}$ 10.7; $J_{d,e}$ 3.3; $J_{d,f}$ 3.9; $J_{e,f}$ 17.5;



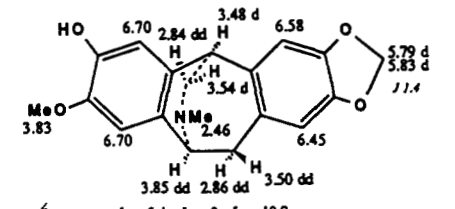
2

$J_{a,b}$ 4.6; $J_{a,c}$ 0; $J_{b,c}$ 11.4; $J_{d,e}$ 3.3; $J_{d,f}$ 3.5; $J_{e,f}$ 17.7;



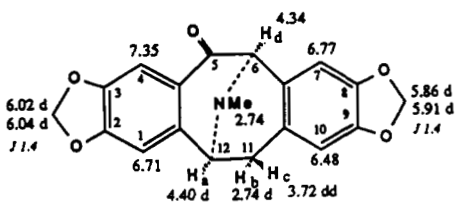
3

$J_{a,b}$ 4.8; $J_{a,c}$ 0; $J_{b,c}$ 11.2; $J_{d,e}$ 3.2; $J_{d,f}$ 3.9; $J_{e,f}$ 17.5;



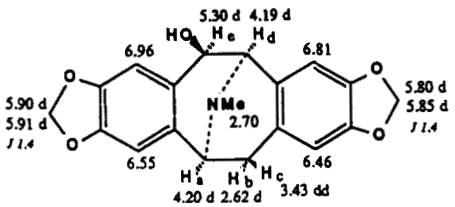
4

$J_{a,b}$ 5.1; $J_{a,c}$ 0; $J_{b,c}$ 10.9; $J_{d,e}$ 3.3; $J_{d,f}$ 3.9; $J_{e,f}$ 17.5;



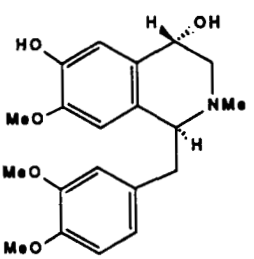
5

$J_{a,c}$ 6.0; $J_{b,c}$ 17.0;



6

$J_{a,c}$ 5.6; $J_{b,c}$ 17.0; $J_{d,e}$ 5.6;



7

hand (3.9 Hz) and H_d and H_e on the other (3.3 Hz). The bridgehead H_d (δ 3.83) shows reciprocating nOe's with both H_f (δ 3.49) and H-1 (δ 6.71), while irradiation of H_e (δ 2.88) leads to an increase in the H-10 (δ 6.50) signal. Additionally, the bridgehead H_a (δ 3.60) displays reciprocating nOe's with the aromatic H-4 and H-7 (δ 6.69 and 6.61), as well as with H_b (δ 2.83) (see Experimental).

It should be pointed out that the aliphatic nmr chemical shifts are generally more downfield for the *N*-nor compound **2** than for the *N*-methyl homologue **1**. A similar trend had previously been observed when the nmr spectra of norbisbenzylisoquinolines were compared with those of the corresponding bisbenzylisoquinoline dimers (11).

From the study of the mass spectra of the isopavine alkaloids that we found in *R. refracta*, we also realized that we had on hand both the known isopavine (−)-reframoline [**4**] (3) and its hitherto unknown structural isomer **3**, here named (−)-refractamine.

Both species underwent substantial loss of 43 atomic mass units (CH_2NMe), characteristic of the mass spectral pattern of isopavines in general, and also showed molecular ion peak *m/z* 325 for the composition $C_{19}H_{19}NO_4$. The base peaks for the two compounds were numerically identical, *m/z* 190, representing *N*-methoisoquinolinium cations with one hydroxyl and one methoxyl substituent on ring A. Furthermore, the ir and uv spectra of isomers **3** and **4** were very close (see Experimental).

The nmr spectral data for (−)-reframoline and the new alkaloid (−)-refractamine are quoted around structures **4** and **3**, respectively. The main difference lies with the two most downfield aromatic protons. In the spectrum of (−)-reframoline [**4**], the H-1 and H-4 absorptions almost overlap at δ 6.70. But the corresponding absorptions in (−)-refractamine [**3**] are well separated and fall at δ 6.74 and 6.67. These assignments were confirmed by an nmr nOe study of (−)-refractamine [**3**], which showed reciprocating enhancements between H-1 (δ 6.74) and H_d (δ 3.83) on the one hand, and between H-4 (δ 6.67) and H_a (δ 3.61), and also between H-4 and 3-OMe (δ 3.83), on the other. CH_2N_2 *O*-methylation of either isomer led to the alkaloid (−)-reframine.

It should be mentioned that refractamine [**3**] was synthesized in the racemic form some fourteen years ago (12). From the low resolution nmr spectra available at that time, however, it was not possible to differentiate purely by spectroscopic means between what we now call refractamine and its structural isomer reframoline. Chromatographic comparisons of the natural (−)-reframoline [**4**] with synthetic racemic refractamine and reframoline were, therefore, also carried out at the time (12).

We immediately suspected that our third new alkaloid, (−)-eschscholtzinone [**5**], $C_{19}H_{15}NO_5$, was unusual because it incorporated five oxygen atoms instead of the usual four. The ir spectrum displayed a prominent conjugated carbonyl absorption at 1680 cm^{-1}. The uv spectrum was more complex than that of a simple pavine or isopavine, with maxima at 236, 276, and 320 nm.

The mass spectrum of (−)-eschscholtzinone [**5**] reinforced our belief that we were indeed dealing with a ketonic species. The molecular ion, *m/z* 337, was also the base peak, but loss of CO from the molecular ion gave rise to an $[M-28]^+$ ion, *m/z* 309 (32%).

The nmr spectrum of (−)-eschscholtzinone has been outlined around structure **5**. The aliphatic region of the spectrum revealed a three-proton spin system (δ 2.74, 3.72, 4.40), a one-proton singlet (δ 4.34), and an *N*-methyl singlet (δ 2.74). The four aromatic singlet absorptions have noticeably different shifts from those of the previously described isopavines, extending as far downfield as δ 7.35. In initial nOe studies, it was noted that irradiation of H_a (δ 4.40) effected an enhancement of only one of the aromatic singlets (δ 6.71). In the isopavines, on the other hand, irradiation of H_a typically enhances two of the aromatic signals. This evidence suggested a pavinoid framework, and structure **5** for (−)-eschscholtzinone was then substantiated by further nOe studies. The *N*-methyl singlet (δ 2.74) was enhanced through irradiation of bridgehead protons H_a or H_d, pointing to the absence of a methylene group in the bridge as found in structures **1**–**4**. Irradiation of H_b (δ 2.74) led to a 35% increase of the aromatic H-10 singlet at δ 6.48 and a 12% increase of the H-1 signal at δ 6.71, a reflection of the overall V-shape of the molecule. Irradiation of H_d (δ 4.34) resulted in a 50% enlargement of the aromatic H-7 signal (δ 6.77), thus identifying all but one of the aromatic protons. The remaining downfield aromatic signal (δ 7.35) could then be assigned H-4, which is adjacent to the ketonic function, thus explaining its downfield shift.

$NaBH_4$ reduction of (−)-eschscholtzinone [**5**] proceeded in stereospecific fashion to afford (−)-eschscholtzinol [**6**], $C_{19}H_{17}NO_5$, λ max (MeOH) 215, 234 sh, and 293 nm (log ε 4.11, 3.78, and 3.89). The mass spectrum of alcohol **6** displayed a strong

molecular ion at *m/z* 339. There was also an ion at *m/z* 321 formed by loss of H_2O from the molecular ion. The base peak, *m/z* 188, represented the 6,7-methylenedioxy-*N*-methoisoquinolinium cation.

The most noticeable changes in the nmr spectrum of (−)-eschscholtzinol [**6**] following D_2O exchange were the upfield shift of the aromatic H-4 at δ 7.35 in **5** to 6.96, and the appearance of a coupling of 5.6 Hz between H_d (δ 4.19) and the newly formed H_e (δ 5.30). This suggested a dihedral angle of approximately 38° between H_d and H_e, consistent with placement of the alcoholic group in the β position. Further support for this conclusion was provided by nOe data. Irradiation of H_e (δ 5.30) led to a sizable enhancement of H_d (δ 4.19) and H-4 (δ 6.96), while the δ 6.81 absorption remained unaffected.

Acetylation of (−)-eschscholtzinol [**6**] using Ac_2O in pyridine gave rise to the corresponding acetate, $C_{21}H_{19}NO_6$, ν max ($CHCl_3$) 1725 cm^{-1}.

Biogenetic considerations induce us to juxtapose the structure of (−)-eschscholtzinone [**5**] by that of (+)-roemecarine [**7**] found in *Roemeria carica* (13, 14) as well as in *R. refracta*. Compound **7** is the first naturally occurring tetrahydrobenzylisoquinoline known to be hydroxylated in ring B. It is possible, therefore, that in vivo oxidation of a close analogue of (+)-roemecarine [**7**] could lead to (−)-eschscholtzinone [**5**]. All the alkaloids described in the present study have negative specific rotations, and incorporate the absolute configurations indicated.

EXPERIMENTAL

PLANT MATERIAL.—*R. refracta* DC. was collected on June 14, 1986, at Bayburt, Gümüshane, Turkey. A voucher specimen, No. 1042, was deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ege University.

EXTRACTION AND FRACTIONATION.—The dried and powdered plant material (16.5 kg) was extracted with EtOH at room temperature to afford the crude extract (1.1 kg). This was taken up in 5% HCl and filtered. The acidic solution was made alkaline with NH_4OH and the alkaloids extracted with $CHCl_3$. Solvent evaporation furnished a crude alkaloidal mixture (20 g). Fractionation was achieved on a column of Kieselgel 60 (70–230 mesh), using $CHCl_3$ gradually enriched with MeOH. Final purification was by Si gel tlc. In some cases, fractions composed of major alkaloids were investigated on a small scale. The main alkaloids were (−)-reframidine, (−)-reframine, (−)-refractamine, (−)-norreframidine, and (−)-reframoline. All ^{1}H-nmr spectra were obtained at 360 MHz in $CDCl_3$ unless indicated otherwise. All compounds are amorphous.

(−)-REFRAMIDINE [**1**].—[α]D −107° (MeOH, *c* = 0.14); uv λ max (MeOH) 216, 234 sh, 246 sh, 297 nm (log ε 4.05, 3.77, 3.52, 3.91); ir ν max ($CHCl_3$) 1615, 1600, 1495, 1475, 1365, 1340, 1220, 1015, 915, 845 cm^{-1}; eims *m/z* $[M]^+$ 323 (31), 322 (42), 281 (8), 280 (42), 279 (10), 222 (6), 189 (13), 188 (100), 165 (6), 164 (6), 163 (12); nmr nOe H-1 to H_d 12%, H_d to H-1 36%, H_d to H_f 5%, H_f to H_d 3%, H_e to H-10 9%, NMe to H_d 13%, H-4 to H_a 22%, H_a to H-4 44%, H-7 to H_a 23%, H_a to H-7 55%, H_a to H_b 21%, H_b to H_a 16%.

(−)-NORREFRAMIDINE [**2**].—[α]D −98° (MeOH, *c* = 0.11); ir ν max ($CHCl_3$) 3350, 1605, 1580, 1495, 1475, 1415, 1365, 1340, 1250, 1190, 1015, 915, 885 cm^{-1}; eims *m/z* $[M]^+$ 309 (18), 308 (26), 293 (6), 281 (20), 280 (100), 279 (34), 250 (5), 223 (5), 222 (15), 174 (53), 165 (13), 164 (11), 163 (21). *N*-Methylation with formaldehyde/$NaBH_4$ at room temperature afforded (−)-reframidine [**1**].

(−)-REFRACTAMINE [**3**].—[α]D −114° (MeOH, *c* = 0.11); uv λ max (MeOH) 230 sh, 242 sh, 293 nm (log ε 3.86, 3.70, 3.91); uv λ max (MeOH + OH^-) 238, 256 sh, 300nm (log ε 3.84, 3.77, 3.92); ir ν max ($CHCl_3$) 3540, 1600, 1495, 1480, 1445, 1420, 1365, 1345, 1340, 1320, 1275, 1240, 1220, 1085, 1020, 915 cm^{-1}; eims *m/z* $[M]^+$ 325 (35), 324 (54), 283 (9), 282 (52), 281 (7), 267 (5), 239 (16), 191 (13), 190 (100), 181 (5), 175 (8), 152 (7); nmr nOe H-4 to 3-OMe 38%, 3-OMe to H-4 60%, H-4 to H_a 23%, H-1 to H_d 51%, H_d to H-1 50%. Treatment of **5** (10 mg) with ethereal CH_2N_2 afforded (−)-reframine (7 mg).

(−)-REFRAMOLINE [**4**].—[α]D −172° (MeOH, *c* = 0.11); uv λ max (MeOH) 230 sh, 294 nm (log ε 3.92, 3.94); uv λ max (MeOH + OH^-) 236, 301 nm (4.01, 3.99); ir ν max ($CHCl_3$) 3540, 1600, 1495, 1475, 1440, 1420, 1360, 1345, 1335, 1290, 1280, 1200, 1085, 1020, 915 cm^{-1}; eims *m/z* $[M]^+$ 325

(28), 324 (42), 283 (8), 282 (37), 281 (11), 267 (5), 239 (15), 191 (13), 190 (100), 181 (4), 175 (8), 152 (5). Treatment of **4** (10 mg) with etheral CH_2N_2 supplied (−)-reframine (7 mg).

(−)-REFRAMINE.—$[\alpha]D$ −123° (MeOH, $c = 0.26$); uv λ max (MeOH) 226 sh, 292 nm (log ε 4.16, 3.93); ir ν max ($CHCl_3$) 1600, 1490, 1475, 1455, 1365, 1330, 1215, 1085, 1020, 915, 840 cm^{-1}; eims *m/z* $[M]^+$ 339 (30), 338 (40), 297 (7), 296 (36), 295 (9), 253 (11), 205 (14), 204 (100), 152 (4); ^1H nmr (200 MHz, $CDCl_3$) δ 2.52 (3H, s, NMe), 2.87 (1H, dd, H_b, $J_{vic} = 4.5$ Hz, $J_{gem} = 10.9$ Hz), 2.91 (1H, dd, H_e, $J_{vic} = 3.3$ Hz, $J_{gem} = 17.7$ Hz), 3.67 (1H, d, H_a, $J_{vic} = 4.5$ Hz), 3.69 (1H, dd, H_f, $J_{vic} = 3.7$ Hz, $J_{gem} = 17.7$ Hz), 3.77 (1H, d, H_c, $J_{vic} = 0$ Hz, $J_{gem} = 10.9$ Hz), 3.86 (3H, s, OMe), 3.87 (3H, s, OMe), 4.05 (1H, dd, H_d, $J_{vic} = 3.3$ and 3.9 Hz), 5.83 and 5.87 (2H, dd, OCH_2O, $J = 1.4$ Hz), 6.50 (1H, s, H-10), 6.62 (1H, s, H-7), 6.74 (1H, s, H-4), 6.78 (1H, s, H-1).

(−)-ESCHSCHOLTZINONE [**5**].—$[\alpha]D$ −122° (MeOH, $c = 0.18$); uv λ max (MeOH) 236, 276, 320 nm (log ε 4.35, 3.84, 3.93); uv λ max (MeOH + H^+) 241, 282, 302 sh, 319 sh, 329, 344 sh nm (log ε 4.33, 3.85, 3.82, 3.89, 3.91, 3.73); ir ν max ($CHCl_3$) 1680, 1615, 1490, 1475, 1375, 1240, 1020, 915 cm^{-1}; eims *m/z* $[M]^+$ 337 (100), 336 (23), 323 (9), 322 (46), 320 (5), 310 (6), 309 (32), 308 (15), 306 (13), 294 (11), 282 (51), 280 (11), 279 (10), 268 (15), 267 (17), 253 (8), 237 (7), 209 (9), 189 (11), 188 (86), 174 (6), 165 (6); nmr nOe H_b to H-10 35%, H_b to H_a 30%, H_b to H-1 12%, H_a to H-1 27%, H-1 to H_a 5%, H_a to NMe 14%, H_d to NMe 8%, H-7 to H_d 20%, H_d to H-7 50%.

(−)-ESCHSCHOLTZINOL [**6**].—Treatment of **5** (8 mg) with $NaBH_4$ in MeOH gave **6** (7 mg): $[\alpha]D$ −150° (MeOH, $c = 0.14$); uv λ max (MeOH) 215, 234 sh, 293 nm (log ε 4.11, 3.78, 3.89); ir ν max ($CHCl_3$) 3420, 1600, 1495, 1475, 1420, 1375, 1365, 1330, 1210, 1095, 1040, 1015, 960, 910, 850, 825 cm^{-1}; eims *m/z* $[M]^+$ 339 (73), 338 (32), 324 (10), 322 (13), 321 (17), 308 (10), 280 (21), 190 (12), 189 (13), 188 (100), 176 (13), 175 (12), 163 (13), 162 (21); nmr nOe H_a to H-1 16%, H_a to NMe 8%, H_d to H-7 27%, H_d to H_e 26%, H_e to H_d 29%, H_e to H-4 20%, H_d to NMe 8%. Acetylation with Ac_2O in pyridine overnight furnished the corresponding acetate ester: $[\alpha]D$ −66° (MeOH, $c = 0.26$); uv λ max ($CHCl_3$) 1725, 1495, 1475, 1420, 1365, 1250, 1220, 1090, 1020, 910, 845 cm^{-1}; eims *m/z* $[M]^+$ 381 (36), 380 (21), 338 (24), 307 (9), 279 (6), 190 (4), 189 (12), 188 (100), 175 (14), 163 (11).

(+)-ROEMECARINE [**7**].—Spectrally and chromotographically identical with that previously reported (13).

ACKNOWLEDGMENTS

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