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NITROTYRASANGUINARINE: AN UNUSUAL NITRATED BENZOPHENANTHRIDINE ALKALOID FROM *HYPECOUM* SPECIES

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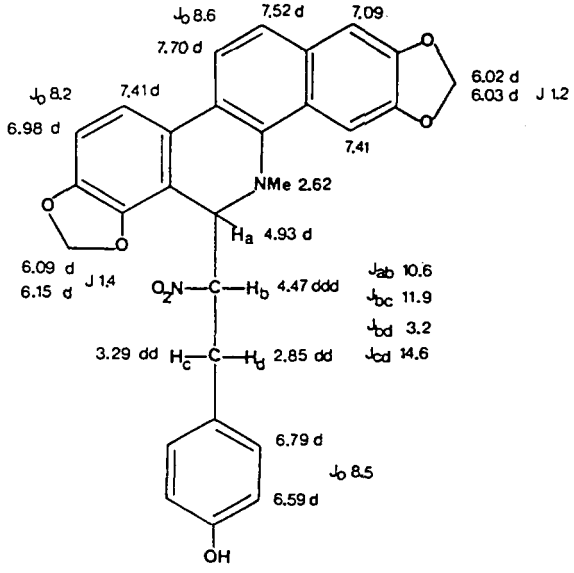
ABSTRACT.—*Hypecoum imberbe*, *Hypecoum procumbens*, and *Hypecoum pendulum* of Turkish origin produce the new benzophenanthridine alkaloid (±)-nitrotyrasanguinarine [**1**].

Until recently, the genus *Hypecoum*, which includes some 15 species distributed from the Mediterranean to Central Asia and Northern China, had been counted as belonging to the family Papaveraceae (1,2). It has now been recognized, however, that *Hypecoum* species belong more appropriately to the botanical family Hypecoaceae (1,2). This family includes only one genus and is intermediate between the Papaveraceae and the Fumariaceae (1).

Protopine is known to be the main alkaloid in *Hypecoum* species (3). In addition, protoberberines (4), secoberbines (4), aporphines (4), spirobenzylisoquinolines (5), isoquinolones (6), and benzophenanthridines (4) are known to be present, besides the structurally uncommon (+)-turkiyenine (7).

Presently, as a result of a study of *Hypecoum imberbe* Sibth. Sm., collected in western Turkey, we have obtained the new and unusual alkaloid (±)-nitrotyrasanguinarine [**1**], $C_{28}H_{22}N_2O_7$, which is the first isoquinoline alkaloid to incorporate an aliphatic nitro group. The occurrence of aromatic nitro groups among the isoquinoline-derived aristolochic acids is, of course, well documented (8).



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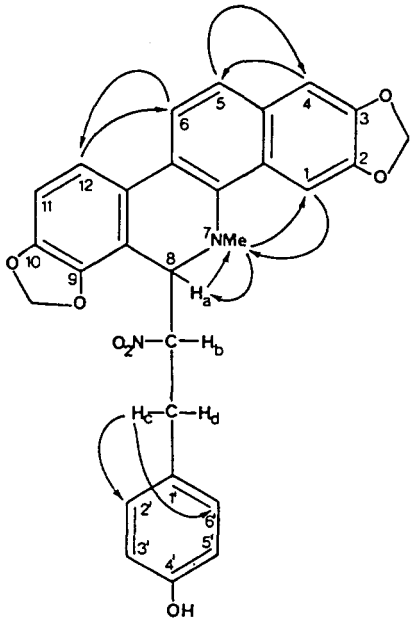

1a

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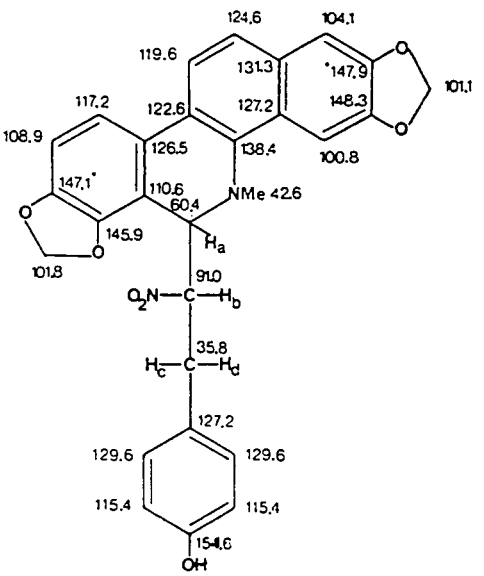
The ^{1}H-nmr spectrum of (±)-nitrotyrasanguinarine has been summarized around expression **1**. The dihydrosanguinarine moiety of the alkaloid was suggested by the presence of two sets of aromatic ortho protons, the first at δ 6.98 and 7.41 and the second at δ 7.52 and 7.70. Two aromatic singlets were also in evidence, at δ 7.09 and 7.41, as well as two methylenedioxy substituents whose protons appeared at δ 6.02 and 6.03 in one case and at 6.09 and 6.15 in the other. An *N*-methyl group was also present, denoted by a 3-proton singlet at δ 2.62.

Very significantly, the H_a (δ 4.93) signal was coupled to H_b (δ 4.47) which was also coupled to H_c (δ 3.29) and H_d (δ 2.85), pointing to the interrelationships among these protons. The H_b signal, relatively downfield at δ 4.47, was consonant with that of a methine proton adjacent to a nitro group as it is known that the methylene protons next to the nitro group of 1-nitropropane fall at δ 4.38 (9). Furthermore, the presence of a para substituted aromatic ring was indicated by two aromatic doublets of two protons each, centered at δ 6.59 and 6.79, sharing an ortho coupling constant of 8.5 Hz.

A complete nmr nOe analysis of (±)-nitrotyrasanguinarine was then carried out, which confirmed the structure assignment. Significant enhancements that throw particular light on those structural features of the alkaloid that were not evident from the spin decoupling studies are indicated in expression **1a**, while more complete nOe data are presented in the Experimental section.

Additional support for structure **1** for (±)-nitrotyrasanguinarine was forthcoming from the ^{13}C-nmr spectrum of the alkaloid. Chemical shift values are given around expression **1b**. These were confirmed first through a GASPE analysis (10), and then by means of a complete COLOC study which describes long range couplings between ^{1}H and ^{13}C atoms (11).

The most intriguing aspect of the ^{13}C-nmr spectrum was the peak at δ 91.0. A signal in this range is unusual, as aliphatic carbons normally appear further upfield, whereas aromatic carbons lie more downfield. It was then found that this signal must be associated with the side chain carbon bearing the nitro group because for 2-nitro-1-butanol the corresponding carbon that is bonded to the nitro group appears at δ 91.7 (12).

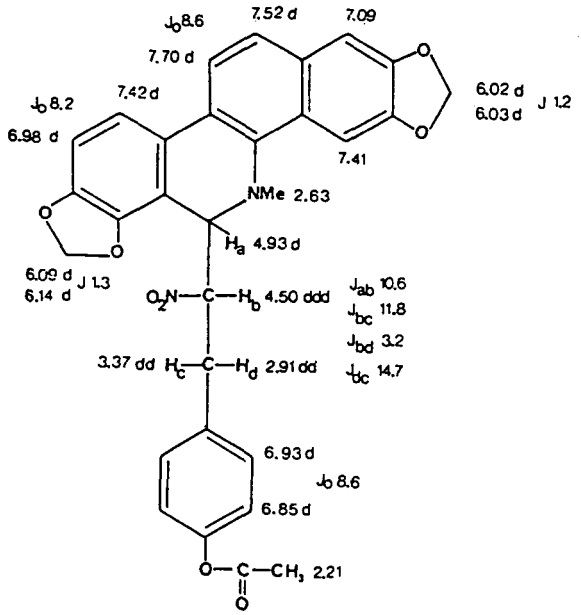


1b

Turning now to the mass spectrum of (±)-nitrotyrasanguinarine, a small molecular ion *m/z* 498 confirmed the formula $C_{28}H_{22}N_2O_7$. Base peak *m/z* 332 was due to loss of the pendant chain from the molecular ion. Another small but significant peak was *m/z* 452 generated through cleavage of the nitro group.

Complementing the above spectral information was the ir spectrum of **1** with absorptions at 1360 and 1550 cm^{-1}, characteristic of the stretching of an aliphatic nitro group (13).

As final proof of structure, (±)-nitrotyrasanguinarine [**1**] was acetylated at room temperature using Ac_2O in pyridine. The ^{1}H-nmr spectrum of the resulting acetate **2**, $C_{30}H_{24}N_2O_8$, was close to that for **1**, except for the acetate singlet at δ 2.21, and the fact that the aromatic protons of the pendant ring had shifted downfield to δ 6.93 and 6.85 as also confirmed by a partial nmr nOe study (see Experimental).



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Additionally, we have found that *Hypecoum procumbens* L. and *Hypecoum pendulum* L., also of Turkish origin, similarly produce (±)-nitrotyrasanguinarine [**1**]. The biogenesis of this alkaloid probably proceeds through initial in vivo oxidation of tyramine (≡ *p*-hydroxyphenethylamine) to 2-(*p*-hydroxyphenyl)-1-nitroethane. The α-nitro carbanion of the latter compound then adds to the commonly occurring alkaloidal salt sanguinarine at the iminium carbon.

As expected, the main alkaloid of *H. imberbe* is protopine (3,4). Other compounds we found to be present are dihydrosanguinarine (4), (+)-turkiyenine (7), (±)-8-methoxydihydrosanguinarine (4), oxyhydrastinine (6), (+)-bulbocapnine (14), ferulamide (15) (ferulamide may actually be an artifact of isolation, formed from the corresponding methyl ester during the alkaloid isolation process), and *N*-(4-hydroxy-3-methoxyphenethyl)ferulamide (16), in addition to the aforementioned (±)-nitrotyrasanguinarine [**1**].

EXPERIMENTAL

PLANT MATERIAL AND ISOLATION.—*H. imberbe* was collected between Salihli and Kula, in Manisa Province. A sample No. 850 was deposited in the herbarium of the Faculty of Pharmacy, Ege University. The powdered plant (18.2 kg) was extracted at room temperature with EtOH. The extract was concen-

trated in vacuo without the application of excessive heat and acidified with 2% HCl. The aqueous solution was made alkaline with NH_4OH, and the alkaloids were extracted with $CHCl_3$. Evaporation of the solvent left a crude extract (28 g) which was placed on a Si gel column. Elution was with $CHCl_3$ gradually enriched with MeOH. Monitoring was by tlc, and fractions of similar composition were combined. Final purification was by tlc on Si gel glass plates. Bands were differentiated under short wave uv light and by means of Dragendorff's reagent. The bands were eluted from the Si gel layer using $CHCl_3$-MeOH (4:1). Compounds isolated were dihydrosanguinarine (5 mg), (+)-turkiyenine (7 mg), protopine (ca. 15 g), (±)-8-methoxydihydrosanguinarine (4 mg), *N*-(4-hydroxy-3-methoxyphenethyl)ferulamide (17 mg), oxyhydrastinine (22 mg), (+ -bulbocapnine (3 mg), ferulamide (16 mg), and (±)-nitrotyrasanguinarine [**1**] (16 mg). All compounds are amorphous.

H. pendulum (7.9 kg dry) was gathered near Uşak (herbarium sample No. 926). It was processed as above to supply 13 mg of (±)-nitrotyrasanguinarine. *H. procumbens* (6.5 kg dry) was obtained near Fethiye in Muğla Province (herbarium sample No. 1050). A workup procedure identical to that described above afforded 8.5 mg of (±)-nitrotyrasanguinarine. All plants were identified by one of the authors (T.G.).

(±)-NITROTYRASANGUINARINE [**1**].—Compound **1**: uv λ max (MeOH) 232, 281, 324 nm (log ε 4.34, 4.31, 3.91); ir ν max ($CHCl_3$) 1360, 1550, 3590 cm^{-1}; eims *m/z* $[M]^+$ 498 (2), 452 (1), 347 (5), 333 (24), 332 (100), 317 (14), 304 (2), 289 (1), 259 (2), 120 (14); hrms calcd for $C_{28}H_{22}N_2O_7$ *m/z* $[M]^+$ 498.1427, found 498.1457. Significant nmr nOe's are H-6 to H-5 (16%), H-6 to H-12 (34%), H-5 to H-6 (13%), H-5 to H-4 (47%), H-12 to H-11 (46%), H-12 to H-6 (77%), H-1 to N-Me (10%), H-4 to H-5 (25%), H-11 to H-12 (13%), H-2′,-6′ to H-3′,-5′ (42%), H-2′,-6′ to H_b (24%), H-3′,-5′ to H-2′,-6′ (45%), H_a to N-Me (10%), H_b to H-5′,-6′ (14%), H_b to H_d (4%), H_c to H_d (29%), H_c to H_a (13%), H_c to H-2′,-6′ (14%), H_d to H_c (32%), H_d to H_b (18%), N-Me to H_1 (51%), N-Me to H_a (32%).

(±)-*O*-ACETYLNITROTYRASANGUINARINE [**2**].—Alkaloid **1** (4 mg) was allowed to stand in a mixture of Ac_2O in pyridine for 10 h. Workup provided **2** (3 mg): ir ν max ($CHCl_3$) 1360, 1550, 1760 cm^{-1}; eims *m/z* $[M]^+$ 540 (3), 452 (1), 366 (7), 332 (100), 317 (15), 304 (4). Important nmr nOe's are H_c to H_d (24%), H_c to H_a (8%), H_c to H-2′,-6′ (10%), H_d to H_c (22%), H_d to H_b (10%).

ACKNOWLEDGMENTS

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