Phytochemistry, Vol 27, No 11, pp 3719–3721, 1988
Printed in Great Britain

0031–9422/88 $3.00+0.00

THE STRUCTURE AND PARTIAL SYNTHESIS OF DELELATINE, AN ALKALOID FROM *DELPHINIUM* SPECIES

Samir A. Ross, Haridutt K. Desai, Balawant S. Joshi, Santosh K. Srivastava, Jan A. Glinski, Si Ying Chen and S. William Pelletier*

Institute for Natural Products Research and the School of Chemical Sciences, The University of Georgia, Athens, Georgia 30602, U.S.A.

(*Received* 1 March 1988)

Key Word Index—*Delphinium elatum*; *D. tatsienense*, Ranunculaceae; delelatine; C_{19}-diterpenoid alkaloid.

Abstract—A new C-19-diterpenoid alkaloid designated as delelatine has been isolated from *Delphinium elatum* L. and *D. tatsienense* Franch. and its structure has been elucidated by spectroanalytical methods. Delelatine has been correlated with 14-acetyl-10-deoxydictyocarpine and synthesized from dictyocarpine, thus confirming its structure and stereochemistry.

INTRODUCTION

The roots of *Delphinium tatsienense* native to Yunnan, China are used in Chinese medicine. Previous work from our laboratory on the constituents of this plant included the isolation of tatsiensine [1], tatsinine [2, 3] and deltatsine [4]. The roots of *D. tatsienense* also contain the C_{19}-diterpenoid alkaloids browniine, deacetylambiguine, delcosine, lycoctonine [1] and the C_{20}-diterpenoid alkaloids ajaconine, dictyzine [5–7], hetisine, and hetisinone [1]. *Delphinium elatum* L. is known to contain delpheline, deltaline, elatine and methyllycaconitine [8].

RESULTS AND DISCUSSION

We wish to report in this paper, the isolation of a polar alkaloid designated as delelatine (**1**) from the seeds of *D. elatum* as well as from *D. tatsienense*. Delelatine was obtained as colourless crystals, mp 84.5–86.5°; $C_{24}H_{37}NO_6$: M^+ *m/z* 435; IR (nujol) 3360 (OH) cm^{-1}; ^1H NMR ($CDCl_3$): δ0.95 (3H, *s*, *tert*-methyl), 1.05 (3H, *t*, $J = 7$ Hz, NCH_2Me), 3.30, 3.40 (each 3H, *s*, OMe), 4.15 [1H, *t*, $J = 4.5$ Hz, C(14)-β-H], 4.22 [1H, *s*, C(6)-α-H], 5.10, 5.20 [each 1H, *s*, O–CH_2–O]. The presence of two methoxyls, an ethyl and a methylenedioxy group in the molecule suggests that delelatine is a new C_{19}-diterpenoid alkaloid. The proton noise-decoupled ^{13}C NMR spectrum showed 22 lines for 24 carbon atoms of the molecule. The SFORD spectrum indicated four quaternary carbons appearing at δ94.0, 81.8, 49.7 and 34.2 that can be readily assigned to C(7), C(8), C(11) and C(4), respectively. The presence of a methylenedioxy grouping at C(7), C(8) was evident from the above data and the methylene carbon signal at δ93.3. As delelatine is a lycoctonine-type alkaloid, the normal oxygen functions at C(1), C(6), C(14) and a methoxyl group at C(16) can be assumed to be present. On the basis of these data and comparison of the ^{13}C NMR spectral assignments of related compounds [8], delelatine probably possesses the partial structure of delpheline in which two of the hydroxyl groups and the remaining methoxyl group should be placed at C(1), C(6) or C(14). Location of the methoxyl group at C(1) was deduced from the mass spectrum, which showed the loss of 31 mass units from the molecular ion [9]. The tentative structure **1** can thus be derived for the new alkaloid.

The structure of delelatine was established by treatment with acetylchloride to afford 14-acetyl-10-deoxydictyocarpine (**2**), a compound previously prepared by deoxygenation of 14-acetyldictyocarpine (**4**) [10]. The structure was confirmed by synthesis from dictyocarpine (**3**), whose structure has been established by an X-ray crystal study [11]. Dictyocarpine was converted to 14-acetyldictyocarpine (**4**) [12]. Treatment of **4** with $SOCl_2$ afforded the corresponding 10-chloro-10-deoxy-derivative (**5**) [10]. Treatment of **5** with tributyltin hydride [13] furnished 14-acetyl-10-deoxydictyocarpine which was identical with delelatine 6,14-diacetate (**2**). Also hydrolysis of **2**, prepared from dictyocarpine via **4** and **5**, in methanolic Sodium hydroxide furnished delelatine (**1**), identical by comparison of mp, TLC, CO-TLC, IR and mass spectrum with natural delelatine. In summary, the structure of delelatine was established by two correlation sequences: **1** →**2**; and **3**→**4**→**5**→**2**→**1**.

EXPERIMENTAL

Mps: corr ^1H NMR spectra were recorded at 90 MHz using TMS as int. standard, ^{13}C NMR spectra were recorded in $CDCl_3$ soln. Mass spectra were determined on a Finnegan Quadrupole 4023 instrument.

Isolation of delelatine (**1**) (a) from *D. elatum*. Seeds of *D. elatum* (450 g) from Harris Moran Seeds were ground, suspended in a mixture of 95% EtOH (1.7 l), H_2O (0.42 l) and hexane (0.52 l), heated under reflux for 2 hr and then vigorously stirred at room temp for 48 hr. The mixture was filtered and the filtrate allowed to stand till the layers separated. Evapn of the hexane extract gave an oil (73.1 g).

The aq ethanolic extract was evapd to give a yellow solid which was partitioned between CH_2Cl_2 (2 × 2.1 l) and 0.75 M H_2SO_4 (2.1 l). Evaporation of the CH_2Cl_2 extract gave a neutral fraction (4.6 g). Basification of the acidic layer (Na_2CO_3, pH 10) and extraction with Et_2O (3 × 2 l) gave a crude alkaloidal fraction (9.6 g). Chromatographic separation (VLC) [14] (9.1 g) on silica gel by elution with hexane and increasing amounts (1–10%) of hexane–EtOH, subsequent purification on a rotor of a Chromatotron (silica gel 60 PF,· 2 mm) and finally sepn on a prep TLC plate (silica gel) afforded delelatine (**1**, 104 mg), mp 84 5–86 5°, $[\alpha]_D^{23\ 5}$ $-27\ 7°$ ($CHCl_3$, *c* 1 05)

(b) from *D tatsienense* Franch The mixture of alkaloids obtained during the isolation of lycoctonine [1] (2 02 g) was chromatographed on 35 g of Al_2O_3 (neutral, grade III) and eluted with toluene with increasing amount of Et_2O The fraction which moved close to lycoctonine was collected (50 mg) and chromatographed twice on prep TLC plates (Al_2O_3) and eluted with toluene 10% MeOH Delelatine (24 mg) was isolated as a gum which crystallized from ether, mp 74°, recrystallization from ether gave material melting at 83 5–84 5° MS, *m/z* (rel int.) 435 $[M]^+$ (2), 404 (25), 403 (100), IR (nujol), 3360, 1160, 1130, 1050, 930 cm^{-1}

^1H NMR (90 MHz, $CDCl_3$) δ0 95 [3H, *s*, C(4)–Me], 1 05 (3H, *t*, $J = 7$ Hz, N–CH_2–Me), 3 30, 3.40 (each 3H, *s*, OMe) 4 15 [1H, *t*, $J = 4.5$ Hz, C(14)–β-H), 4 22 [1H, *br s*, C(6)-α-H], 5 10, 5 20 (each 1H, *s*, O–CH_2–O), ^{13}C NMR ppm 83 9 C(1), 26 3* C(2), 36 8 C(3), 34.2 C(4), 55 9 C(5), 78 8 C(6), 94 0 C(7), 81 8 C(8), 47 8 C(9), 42 6 C(10), 49 7 C(11), 27 0* C(12), 36 5 C(13), 73 9 C(14), 32 2 C(15), 81 8 C(16), 63 9 C(17), 25 3 C(18), 57 5 C(19), 50 6 N–$\underline{C}H_2$Me, 14 0 N–$CH_2\underline{Me}$, 56 4 C(1) OMe, 56 4 C(16) OMe, 93 2 O–CH_2–O (*these assignments may be interchanged)

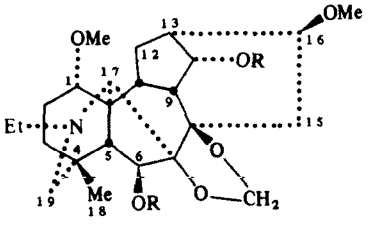
Conversion of deleletine (**1**) *to delelatine* 6,14-*diacetate* (**2**) Delelatine (30 mg) was stirred with MeCOCl (1 ml) at 25° for 24 hr Evapn *in vacuo* gave a residue that was chromatographed on neutral Al_2O_3 (Act III) to afford **2** (32 mg), $[\alpha]_D^{23}$ $-62\ 4°$ ($CHCl_3$; *c* 0 62) EIMs *m/z* 519 (M^+, $C_{28}H_{41}NO_8$) The ^1H and ^{13}C NMR spectra were identical with those of an authentic sample [10]

14-*Acetyldictyocarpine* (**4**) Dictyocarpine (**3**) was acetylated in the usual manner to afford **4**, identical with an authentic sample by comparison of the ^1H and ^{13}C NMR spectra [8, 12]

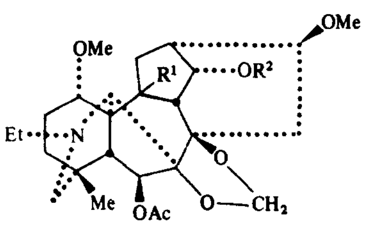
14-*Acetyl*-10-*chloro*-10-*dehydroxydictyocarpine* (**5**) 14-Acetyldictyocarpine (**4**, 110 mg) was dissolved in dry benzene (7 ml), treated with freshly dist $SOCl_2$ (1 5 ml) and stirred at room temp for 24 hr The solvent was removed *in vacuo* and the residue purified on a Chromatotron (Al_2O_3) to afford **5** (110 mg) The properties agreed with those of an authentic sample [10]

14-*Acetyl*-10-*deoxydictyocarpine* (**2**) *from* **5** A soln of the chloro compound (**5**, 101 mg) in dry C_6H_6 (25 ml) was heated under reflux in a N_2 atm with a soln of tributyltin hydride (250 mg in 2 ml C_6H_6) for 16 hr An additional quantity of tributyltin hydride (110 mg in 2 ml C_6H_6) was added and the heating continued for 4 hr The solvents were removed under red pres and the oily residue purified with a Chromatotron successively on Al_2O_3 and silica gel plates The first band that eluted with hexane 25% Et_2O (silica gel rotor) gave the starting material **5**, 37 mg The major band that eluted with hexane Et_2O (1 1) afforded (**2**) as an amorphous compound (61 mg) The ^{13}C NMR spectrum ($CDCl_3$) was identical with that of an authentic sample [10]

Hydrolysis of 14-*acetyl*-10-*deoxydictyocarpine* (**2**) *to delelatine* (**1**) A solution of (60 mg) of **2**, prepared from dictyocarpine via **4** and **5**, in MeOH (13 ml) was stirred with 10% NaOH (1 ml) at room temp for 50 hr The progress of the hydrolysis was monitored by TLC Usual work-up and extraction with $CHCl_3$ (30 ml × 5) gave a residue (45 5 mg) that was purified on a silica gel rotor of a Chromatotron by elution with Et_2O 0 1% MeOH to give colourless crystals of **1**, mp 75–77° (25 mg), recrystallization gave a sample with mp of 84–86° (17 mg) Identity with authentic delelatine was confirmed by comparison of its TLC, co-TLC, IR and ^{13}C NMR spectral properties



1 R = H Delelatine
2 R = Ac



3 R^1 = OH, R^2 = H
4 R^1 = OH, R^2 = Ac
5 R^1 = Cl, R^2 = Ac

Acknowledgements—We thank Mr Courtney Pape for the mass spectra We acknowledge with gratitude, financial support of this work by the American Cyanamid Company

REFERENCES

1 Pelletier, S W, Glinski, J A, Joshi, B S and Chen, S Y (1983) *Heterocycles* **30**, 1347
2 Glinski, J A., Joshi, B S, Chen, S Y and Pelletier, S W (1984) *Tetrahedron Letters* **25**, 1211
3 Maddry, J A, Newton, G M and Pelletier, S W (1986) *J Nat. Prod* **49**, 674
4. Joshi, B S, Glinski, J A, Chokshi, H P, Chen, S Y, Srivastava, S K and Pelletier, S W (1984) *Heterocycles* **22**, 2037
5 Tashkhadzhaef, B (1979) *Khim Prir Soedin* 812
6 Salimov, B J, Tashkhadzhaef, B and Yunusov, M S (1981) *Khim Prir Soedin* 86
7. Joshi, B S, Wunderlich, J K and Pelletier, S W (1987) *Can J Chem* **65**, 99
8 Pelletier, S W, Mody, N V., Joshi, B S and Schramm, L C (1984) in *Alkaloids Chemical and Biological Perspectives* Vol 2, Ch 5 Wiley, New York
9 Yunusov, M S, Rashkes, Y V, Tel'nov, V A and Yunusov, S Y (1969) *Khim Prir Soedin* 515
10 Kulanthaivel, P and Pelletier, S W (1988) *Tetrahedron* **44**, 000
11 Pelletier, S W, Varughese, K I (1984) *J Nat Prod* **47**, 643
12 Pelletier, S. W, Dailey Jr O D, Mody, N V and Olsen, J D (1981) *J Org Chem* **46**, 3284

13 Kulanthaivel, P. and Pelletier, S W. (1987) *Tetrahedron Letters* **28**, 3883.

14. Pelletier, S W, Chokshi, H P and Desai, H K (1986) *J. Nat Prod.* **49**, 892

Phytochemistry, Vol. 27, No 11, pp 3721–3723, 1988
Printed in Great Britain

0031–9422/88 $3 00+0 00

15β-HYDROXYVINCADIFFORMINE, AN ALKALOID FROM THE LEAVES OF *RHAZYA STRICTA*

ATTA-UR-RAHMAN*, TALAT FATIMA and SAJIDA KHANUM

H. E. J Research Institute of Chemistry, University of Karachi, Karachi 32, Pakistan

(*Received in revised form* 24 *February* 1988)

Key Word Index—*Rhazya stricta*, Apocynaceae, alkaloid; 15β-hydroxyvincadifformine, homodecoupling; ^{13}C NMR, DEPT

Abstract—A new alkaloid, 15β-hydroxyvincadifformine has been isolated from the leaves of *R. stricta*.

INTRODUCTION

Rhazya stricta (Decaisne) is a small glabrous erect shrub, abundantly distributed in Pakistan [1–3]. The plant is well known in the indigenous system of medicine for the treatment of various diseases [4–7]. Extracts of *R. stricta* showed anti-cancer and antineoplastic activity [8–11] We have previously reported a number of new alkaloids from the plant [4, 12–21]. In continuation of our studies on the isolation and structure elucidation of new chemical constituents from the leaves of *Rhazya stricta*, we have isolated a new alkaloid, 15β-hydroxyvincadifformine (**1**), the structure of which has been established by spectroscopic studies.

RESULTS AND DISCUSSION

The crude alkaloidal material obtained from the ethanolic extract of the fresh leaves of *Rhazya stricta* by conventional procedures [4, 5] was subjected to column chromatography for preliminary fractionation The fraction obtained on elution with petrol–chloroform (1:3) afforded a mixture of alkaloids. This mixture was subjected to repeated chromatographic purification on silica gel (Merck, GF-254) in petrol–chloroform–methanol (14:5.1). The faster moving alkaloid was obtained as a pale yellow amorphous material and gave a UV spectrum characteristic of the anilinoacrylate system. The IR spectrum showed peaks at 3420 (–NH), 3400 (OH) and 1690 cm^{-1} (conjugated ester· –N–C=C–CO$_2$Me). The HRMS afforded M at 354 1943 ($C_{21}H_{26}N_2O_3$), indicating the presence of 10 double bond equivalents in the molecule and a fragmentation characteristic of the Aspidosperma skeleton. The ^{1}H NMR spectrum (CDCl$_3$, 300 MHz) showed the presence of 26 protons. A three-proton singlet at δ3.75 was assigned to the ester methyl protons. Another three proton singlet at δ0.67 ($J_{18,19} = 7.5$ Hz) was assigned to the methyl protons of the ethyl group. Two quartets resonated at δ0.96 ($J_{19\alpha,18} = 7.5$ Hz) and δ1.11 ($J_{19\beta,18} = 7.5$ Hz) for the C-19 methylene protons of the ethyl group, their separate chemical shifts being on account of their prochiral nature. Such a differentiation between the two methylene protons of the ethyl group has previously been observed in vindoline [22] and bannucine [23]. A multiplet for C-15α H appeared at δ3.74 Other chemical shift assignments for 15β-hydroxyvincadifformine are shown in Table 1.

Two dimensional NMR measurements (COSY-45, 2D *J*-resolved) were carried out to verify the assignments. The coupling interactions were established through COSY-45 spectrum while the multiplicities of the overlapping proton signals were determined from the 2D *J*-resolved spectrum. The assignments for the C-18 methyl protons at δ0 66 could thus be confirmed from the COSY-45 spectrum, which showed strong cross peaks with the signals at δ0.99 for C-19Hα and at δ1.11 for C-19H β protons. The signal at δ3.74 (C-15H α) showed strong cross peaks with the signal at δ2.01 (C-14α), while the signal at δ2.23 (C-5) showed cross peaks with the signal at δ1.74 C-6 protons The COSY-45 spectrum of 15β-hydroxyvincadifformine (**1**) is presented in Fig. 1 with the important interactions indicated.

The ^{13}C NMR spectrum (CDCl$_3$, 75 MHz, DEPT) of the compound showed a downfield signal at δ169.30 for the ester carbonyl group. Another downfield quaternary signal at δ167.23 was assigned to the C-2 carbon atom. A downfield signal for the hydroxyl-bearing C-15 methine carbon resonated at δ70.51, its downfield chemical shift