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THE PRINCIPAL ALKALOIDS OF *DELPHINIUM MACROCENTRUM* FROM MT KENYA

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Key Word Index—*Delphinium macrocentrum*; Ranunculaceae; diterpenoid alkaloids; desacetylnudicauline; macrocentridine; macrocentrine.

Abstract—An investigation of the alkaloids of *Delphinium macrocentrum* from Mt. Kenya resulted in the isolation and identification of the principal components as the known bases browniine, delcosine, delcosine 14-acetate, deltatsine, methyllycaconitine, and hetisine 13-*O*-acetate plus three apparently previously undescribed compounds which have been named desacetylnudicauline, macrocentridine and macrocentrine, and for which structures were established.

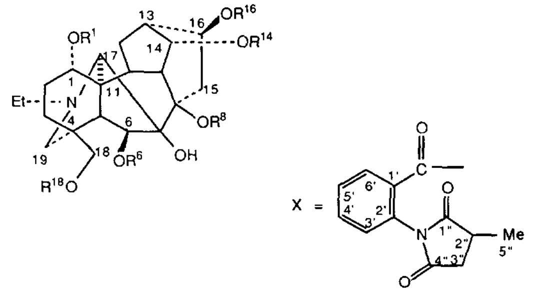
INTRODUCTION

Plants of the genus *Delphinium* have a circumpolar distribution in the Northern Hemisphere but, apart from introduced species, only penetrate into the Southern in Africa. Although numerous studies of species from Asia, Europe and N. America have revealed them to be a rich source of diterpenoid alkaloids [1], those from Africa have received little attention. For this reason we seized an opportunity to examine *D. macrocentrum* Oliv., a native of the highlands of Kenya and other parts of East Africa [2], near the southernmost limit of the natural range of the genus. No previous chemical investigations of this plant had been reported*.

RESULTS AND DISCUSSION

In line with its northern relatives, the air-dried epigeal parts of *D. macrocentrum* collected on Mt Kenya proved to contain *ca* 0.3% of a mixture of alkaloids. Extensive chromatographic fractionation of this mixture (see Experimental) yielded nine major components homogeneous, or nearly so, by TLC analysis which were then characterized spectroscopically (MS, IR, ^{1}H and ^{13}C NMR). This resulted in the identification of five of them as the known [3] C_{19}-diterpenoid alkaloids browniine (**1**), delcosine (**2**), delcosine 14-acetate (**3**), deltatsine (**3**), and methyllycaconitine (**5**); while another was hetsine 13-*O*-acetate (**6**) [4] a C_{20}-diterpenoid. The other alkaloids (**A**–**C**) did not appear to have been described before.

Alkaloid **A**, obtained as an amorphous solid, was first recognised as a congener of **5** on the basis of its ^{1}H NMR spectrum (see Experimental) which contained *inter alia* signals for an anthranoyl unit, as well as an *N*-ethyl and three methoxyl groups. The ^{13}C NMR spectrum had absorptions for 36 carbons and as the MS revealed an apparent molecular ion at *m/z* 668 we formulated the alkaloid as $C_{36}H_{48}N_2O_{10}$. This corresponded to **5** in which one of the methoxyl groups was replaced by hydroxyl. One such possibility was the known alkaloid glaudelsine (**7**) [3, 5] but the ^{13}C NMR spectrum of **A** although very similar to that of **7** showed some differences (see Table 1). In particular, whereas **7** was reported to shown resonance at δ 84.9 (*d*) and 37.0 (*t*) ppm each corresponding to two carbons, the spectrum of **A** contained absorptions at those positions for single carbons and had two additional signals at δ 37.8 (*s*) and 75.2 (*d*) ppm. The δ 84.9 and 75.2 ppm absorptions for **A** may also be compared with the single resonance at δ 83.9 (*d*) for C-1



1 $R^1 = R^6 = R^{16} = R^{18} = $ Me , $R^8 = R^{14} = $ H
2 $R^1 = R^8 = R^{14} = $ H, $R^6 = R^{16} = R^{18} = $ Me
3 $R^1 = R^8 = $ H, $R^6 = R^{16} = R^{18} = $ Me , $R^{14} = $ Ac
4 $R^1 = R^{14} = $ H, $R^6 = R^8 = R^{16} = R^{18} = $ Me
5 $R^1 = R^6 = R^{14} = R^{16} = $ Me , $R^8 = $ H, $R^{18} = $ X
7 $R^1 = R^{14} = R^{16} = $ Me , $R^6 = R^8 = $ H, $R^{18} = $ X
8 $R^1 = R^6 = R^{16} = $ Me , $R^8 = R^{14} = $ H, $R^{18} = $ X
9 $R^1 = R^6 = R^{16} = $ Me , $R^8 = R^{14} = R^{18} = $ H
10 $R^1 = R^6 = R^{16} = $ Me , $R^8 = $ H, $R^{14} = $ Ac, $R^{18} = $ X
11 $R^1 = R^8 = R^{14} = R^{16} = $ H, $R^6 = R^{18} = $ Me
12 $R^1 = R^6 = R^{14} = R^{18} = $ Me , $R^8 = R^{16} = $ H

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*A preliminary report of our work was made at the First Princess Congress of Natural Products Chemistry, Bangkok, December 1987.

Table 1. Carbon-13 chemical shifts and assignments for desacetylnudicauline (**8**), glaudelsine (**7**), methyllycaconitine (**5**) and macrocentridine (**11**)

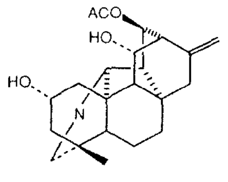
C	**8**	**7**	**5**	**11**
1	84.9	84.9	83.9	72.6
2	25.3	25.3	26.0	27.5
3	32.1	32.2	32.0	29.3
4	37.8	37.0	37.6	37.6
5	45.1	45.8	43.2	43.9
6	90.3	90.3	90.8	89.9
7	89.2	89.2	88.5	87.8
8	76.3	76.3	77.4	78.4
9	50.1	50.2	50.3	45.1
10	36.3	37.9	38.0	43.1
11	48.3	48.3	49.0	48.5
12	27.4	27.6	28.7	28.7
13	46.0	46.1	46.1	45.1
14	75.2	84.9	83.9	76.1
15	33.1	33.1	33.6	38.7
16	81.7	81.7	82.5	72.3
17	65.0	65.0	64.5	66.1
18	69.4	69.5	69.5	77.2
19	52.3	52.4	52.3	57.0
NCH_2Me	51.2	51.2	50.9	50.4
	14.2	14.3	14.0	13.7
MeO-1	56.1	56.1	55.7	47.4
6	58.3	—	57.8	—
14	—	58.3	58.2	—
16	56.5	56.5	56.3	—
18	—	—	—	59.1
C=O	164.2	164.2	164.1	—
1′	127.0	127.1	127.1	—
2′	133.1	133.1	133.1	—
3′	129.4	129.5	129.4	—
4′	133.7	133.7	133.6	—
5′	131.0	131.0	131.0	—
6′	130.0	130.1	130.0	—
1″	179.9	179.8	179.8	—
2″	37.0	37.0	37.0	—
3″	35.2	35.3	35.3	—
4″	175.9	175.9	175.8	—
5″	16.3	16.3	16.4	—

and C-14 in **5** (see Table 1). The EIMS fragmentation pattern of **A** revealed the ion at *m/z* 637 [M − 31] to be considerably more abundant than that at 651 which is in accord with a methoxyl group at C-1 [3, 6]. Thus we deduced **A** to have the structure **8**. Consistent with this, saponification of **A** yielded a carbinolamine whose ^1H and ^{13}C NMR spectra were in agreement with those reported for delectinine [3, 7] (**9**); while acetylation with acetic anhydride in pyridine, a reagent which in the case of lycoctonine -type alkaloids leaves the tertiary hydroxyls at C-7. C-8 unesterified, yielded a product identical (^1H NMR, TLC) with nudicauline (**10**) [8]. Thus **A** is desacetylnudicauline (**8**).

Alkaloid **B** was also obtained as an amorphous solid. Its high resolution EIMS revealed the molecular composition to be $C_{23}H_{37}NO_7$. As the ^1H NMR spectrum contained absorptions due to an *N*-ethyl and two methoxyl groups, we suspected this alkaloid to belong to the C_{19} lycoctonine-series with two methoxyl and five hydroxyl groups probably distributed over the usual sites of oxygenation: 1α, 6β, 7, 8, 14α, 16β, 18. The ^{13}C NMR spectrum of **B** contained resonances at δ 78.4 (*s*) and 87.8 (*s*) ppm which could be assigned to C-7 and 8, each carrying a hydroxyl group [3]. Another absorption at δ 77.2 (*t*) ppm was similarly characteristic of a methoxylated C-18 [3], while 6β-methoxylation was indicated by a resonance at δ 89.9 (*d*) ppm [3]. Location of a methoxyl at C-1 had been considered to be unlikely since the abundance of the [M − 31] fragmentation was less than that of the [M − 17]. As well the high-field methylene -carbon resonance at δ 27.5 and 29.3 assigned to C-2 and C-3 are as expected for a 1α-hydroxylated lycoctonine [3]. Placement of another hydroxyl at C-14 was also indicated by the ^{13}C NMR spectrum which contained an absorption at δ 76.1(*d*) ppm, but none in the region δ 83.5–85.0 ppm seen when this carbon is α-methoxylated [3].

There then remained the location of one hydroxyl group. Most lycoctonine-type alkaloids are 16β-methoxylated and this is associated with a methine ^{13}C-resonance at 79.5–84.5 ppm [3]. As required by our previous assignment of the methoxyls to C-6 and 18, alkaloid **B** exhibited no such absorption. We tentatively considered that it was 16β-hydroxylated and assigned the methine signal at δ 72.3 (*d*) ppm to this centre. We thus arrived at structure **11** for alkaloid **B**, which we have named macrocentridine.

Macrocentridine may be viewed as a biosynthetic precursor of delcosine: *O*-methylation of the 16β-hydroxyl generates **2**; and this was the basis for the location of the group at this centre and the assignment of stereochemistry. Further support for the inter-relationship of macrocentridine and delcosine was provided by their EIMS. A prominent ion at *m/z* 264 in the MS of **2** has been ascribed [9] to a fragment in which the C/D ring system together with C-14 and 16 substituents is retained. As required by this hypothesis, in the MS of **11** an analogous ion appeared at *m/z* 250. Macrocentridine is



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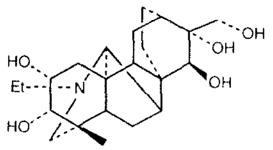

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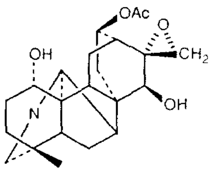

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the second example of a C_{19} lycoctonine-type alkaloid carrying a hydroxyl at C-16, the only other being delbiterine (**12**) [3, 10] for which, unfortunately, the ^{13}C NMR spectrum has not been published.

Alkaloid C, which we have called macrocentrine, crystallized as a hydrate. The derivation of its structure as the unusual C_{20}-diterpenoid **13** was communicated previously [11], and we only note here that while it appears likely to have arisen by the hydrolytic cleavage of a 16α,17-epoxy precursor this does not seem to have taken place during the processing of the plant or fractionation of the alkaloids, for under the same experimental conditions, we were able to isolated an epoxide analogue **14** from *Aconitum delphinifolium* DC. [14]. Thus while **13** might be an artefact of isolation, involving the hydrolysis of **14**, we think that this is unlikely to have occurred to any great extent.

EXPERIMENTAL

All TLC was on silica gel (Merck) using $CHCl_3$–MeOH (5:1) as eluant, and I_2 for detection. The ^{13}C NMR spectra were measured at 50.3 MHz, using $CDCl_3$ as solvent unless otherwise specified. Concordance of the data with that for known alkaloids means that all chemical shifts were within ± 0.2 ppm of the reported values. ^1H NMR spectra were of solutions in $CDCl_3$, at 200 MHz. All shifts (δ) are in ppm relative to TMS = 0.

The epigeal parts of *Delphinium macrocentrum* Oliv. were collected along the Sirmon Track (*ca* 3000 m) on Mt Kenya in August, 1986 just prior to blossoming. The plants were identified by Mr S. G. Mathenge, Curator of the Herbarium, University of Nairobi, and a voucher specimen is deposited in the Herbarium of the University of Calgary.

The air-dried and finely ground plant material (665 g) was repeatedly extracted with 95% aq. EtOH in a Waring blendor until the filtered extracts were almost colourless. The filtrates were combined and concd under red. pres. (cyclone evaporator) to a dark green syrup (*ca* 100 ml). This was then partitioned between $CHCl_3$ (500 ml) and aq. H_2SO_4 (0.5 M 500 ml). The $CHCl_3$ phase was further extracted with aq. H_2SO_4 (5 × 300 ml) until the last aq. wash showed only a very faint opalescence when tested with Mayer's reagent.

Some crushed ice was added to the aq. extracts which were then brought to pH 7 (indicator paper) with satd aq. Na_2CO_3 and extracted with $CHCl_3$ (3 × 200 ml), after which more alkali was added to bring it to pH 11 and the $CHCl_3$ extraction repeated (2 × 200 ml). The $CHCl_3$ extracts were dried ($MgSO_4$) and evapd to yield the crude alkaloids (2.8 g).

This material was dissolved in $CHCl_3$ (50 ml) and then extracted with aq. H_2SO_4 (5 × 150 ml), until the final extract gave only a very weak reaction with Mayer's reagent. The combined acid extracts were basified as before to pH 5, 7, 9, and 11 and extracted at each of these pH-points with $CHCl_3$ (3 × 100 ml). The individual groups of extracts were dried ($MgSO_4$) and evaporated to yield the purified mixed alkaloids: I (pH 5, 1.15 g), II (pH 7, 0.36 g), III (pH 9 0.43 g) and IV (pH 11 0.02 g). TLC analysis revealed 4 major and 3 minor components in I, 2 major and 2 minor in II, while III and IV were similar, sharing two major components.

Separation of the components of I was carried out by conventional gravity-feed column chromatography (CC) over alumina (Woelm neutral, activity III), or by short-column vacuum liquid chromatography (VCC) [12] over alumina (Merck 60 HF-254, basic, type E). The resolution achieved by CC was equivalent to that obtained by VCC, but the latter gave a faster separation. The eluants used in both cases were a sequence of toluene, increasing amounts of $CHCl_3$ in toluene, $CHCl_3$, increasing amounts of MeOH in $CHCl_3$, and finally MeOH [13]. Individual fractions were analysed by TLC and pooled as appropriate. Where necessary, further purification was achieved by PTLC. By these means nine major components were isolated, homogeneous by TLC or almost so. Of these five were identified by the concordance of their ^{13}C and ^1H NMR spectra with those reported for the authentic alkaloids [4] as browniine (*ca* 10% of I), delcosine (*ca* 30% of I), delcosine 14-*O*-acetate (*ca* 10% of I), deltatsine (*ca* 10% of I), and methyllycaconitine (*ca* 25% of I). Two other bases appeared to be previously undescribed. They had the following properties.

Alkaloid **A** (*Desacetylnudicauline*). Amorphous off-white solid (*ca* 15% of I) R_f 0.7; EIMS *m/z* 668 (2), 651 (14), 638 (13), 637 (26), 635 (13), 583 (15), 436 (15), 216 (22), 120 (20), 71 (26), 43 (100); ^1H NMR ($CDCl_3$) δ 1.06 (3H, *t*, J = 7.2 Hz, $MeCH_2N$), 3.25 (3H, *s*, OMe) 3.36 (6H, *s*, 2 × OMe), 7.30 (1H, *dd*, J = 7.5, 2 Hz), 7.57 (1H, *dt*, J = 7.5, 2 Hz), 7.72 (1H, *dt*, J = 7.5 2 Hz), 8.06 (1H, *dd*, J = 7.5, 2 Hz); ^{13}C NMR see Table 1; $[\alpha]_D^{23}$ + 27° (MeOH; *c* 0.6).

Saponification of **A**. To a solution of **A** (46 mg) in MeOH (5 ml) was added aq. NaOH (1 ml, 10% w/v) and the mixture was kept at room temp. for 24 hr. The solvent was removed under red. pres. and the residue partitioned between H_2O (*ca* 2 ml) and $CHCl_3$ (3 × 2 ml). The $CHCl_3$ extracts were combined, dried ($MgSO_4$) and evapd to yield a colourless glass (30 mg) which refused to crystallize but which was homogeneous upon TLC analysis and had ^1H and ^{13}C NMR spectra as reported for delectinine [3, 7].

Acetylation of **A**. A solution of **A** (20 mg) in Ac_2O (0.5 ml) and pyridine (0.5 ml) was kept at room temp. for 48 hr. After removing the bulk of the solvents under high vacuum at room temp., the residue was dissolved in $CHCl_3$ and washed with aq. H_2SO_4, then aq. $KHCO_3$. The $CHCl_3$ was dried ($MgSO_4$) and evaporated to yield a colourless glass with a ^1H NMR spectrum identical to that of nudicauline and cochromatographing with that alkaloid upon TLC.

Alkaloid **B** (*macrocentridine*). An amorphous off-white solid (*ca* 7% of I); R_F 0.4; EIMS *m/z* 439.2569 (10) (calcd for $C_{23}H_{37}NO_7$ 439.2570), 424 (80), 422 (64), 408 (53), 406 (60), 30 (50), 250 (20), 114 (25), 108 (25), 91 (38), 71 (32), 58 (100), 45 (83): IR ν_{max} ($CHCl_3$) 3433 (vs); ^1H NMR ($CDCl_3$) δ 1.09 (3H, *t*, J = 7.2 Hz $MeCH_2N$), 3.33 (3H, *s*, OMe), 3.39 (3H, *s*, OMe) 4.28 (1H, *t*, J = 5 Hz H-14β); ^{13}C NMR as in Table 1; $[\alpha]_D^{23}$ + 27° (EtOH; *c*.1.0).

Similar fractionation of II yielded browniine and macrocentrine as the major alkaloids, while III and IV gave macrocentrine [11], and the known alkaloid 13-*O*-acetylhetisine [4].

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REFERENCES

1. Pelletier, S. W. and Page, S. W. (1986) *Nat. Prod. Rep.* **3**, 451
2. Turril, W. B. and Milne-Redhead (eds) (1952) *Flora of Tropical East Africa*, No. 154, *Ranunculaceae*, pp. 19–22. Crown Agents, London.
3. Pelletier, S. W., Mody, N. V., Joshi, B. S. and Schram, L. C. (1984) in *Alkaloids: Chemical and Biological Perspectives*, Vol

2, (Pelletier S. W., ed.), p. 205. Wiley-Interscience, New York.

4. Benn, M., Richardson, J. F. and Majak, W. (1986) *Heterocycles*, **24**, 1605.
5. Pelletier, S. W., Dailey Jr., O. D. and Mody, N. V. (1981) *J. Org. Chem.* **46**, 3284.
6. Yunusov, M. S., Rashkes, Ja. V., Telnov, V. A. and Yunusov, S. Yu. (1969) *Khim. Prir. Soedin.* **5**, 515.
7. Pelletier, S. W. and Sawhney, R. S. (1978) *Heterocycles* **9**, 463.
8. Kulanthaivel, P. and Benn, M. H. (1985) *Heterocycles* **23**, 2515.
9. Sastry, S. D. in Waller, G. R. (Ed.) (1972) *Biochemical Applications of Mass Spectrometry*, p 655. Wiley Interscience, New York.
10. Salimov, B. T., Yunusov, M. S. and Yunusov, S. Yu. (1978) *Khim. Prir. Soedin.* 106.
11. Benn, M. H., Okanga, F., Richardson, J. F. and Munavu, R. M. (1987) *Heterocycles* **26**, 2331.
12. Pelletier, S. W., Joshi, B. S. and Desai, H. K. (1985) in *Advances in Medicinal Plant Research* (Vlietinck, A. J. and Dommisse, R. A., eds), p 153. Wissenschaftliche Verlagsgesellschaft, Stuttgart.
13. Okanga, F. I. (1987) M. Sc. Thesis. University of Calgary.
14. Kulanthaivel, P. and Benn, M. H. (1988) *Phytochemistry* **27**, 3998.