Pergamon

0031-9422(94)00827-2

Phytochemistry, Vol. 38, No. 6, pp. 1533–1535, 1995

0031-9422/95 $9.50 + 0.00

O-METHYLODULINE AND *N*-DEMETHYLMASONINE, ALKALOIDS FROM *NARCISSUS PSEUDONARCISSUS*

MIRKO KREH and RUDOLF MATUSCH*

Institut für Pharmazeutische Chemie der Philipps-Universität Marburg, Marbacher Weg 6, D-35032 Marburg, Germany

(*Received* 25 *July* 1994)

Key Word Index—*Narcissus pseudonarcissus*; Amaryllidaceae; daffodil bulbs; alkaloids; *O*-methyloduline; *N*-demethylmasonine; oduline; masonine; NMR.

Abstract—*O*-Methyloduline and *N*-demethylmasonine, two so far unknown Amaryllidaceae alkaloids, have been found in bulbs of *Narcissus pseudonarcissus*. Their structures were established by NMR spectroscopy (COLOC and NOE experiments). Complete spectral data for oduline and masonine are also given. It is shown, that *O*-methyloduline is not an artefact of plant extraction with methanol.

INTRODUCTION

Narcissus pseudonarcissus subsp. *pseudonarcissus* cv. Carlton is a typical large-cupped daffodil of garden origin. Although this daffodil is used in very high quantities as a cut flower, as well as a garden plant [1], phytochemical investigations are rare. Only the isolation of the alkaloid, narciclasine, has been published [2]. In the present paper, we report the isolation of several alkaloids from this species of daffodil. Two of them, *O*-methyloduline (**1**) and *N*-demethylmasonine (**2**), are reported for the first time as natural products. In addition, we identified the known alkaloids, lycorenine (**3**), oduline (**4**), hippeastrine, masonine (**5**), homolycorine (**6**), haemanthamine, vittatine, galanthamine, *N*-demethyl-galanthamine, lycoramine, epi-*N*-demethyllycoramine, narwedine and *O*-methyllycorenine (**7**).

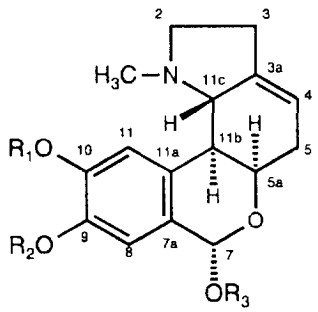
RESULTS AND DISCUSSION

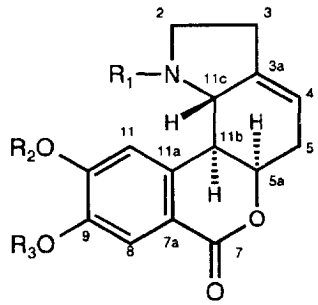
Alkaloids were isolated from air-dried bulbs by extraction with methanol and routine acid-base work-up procedures (see Experimental). From fraction VII-4, we isolated **1** together with oduline (**4**). The EI-mass spectrum of **1** indicated an alkaloid of the lycorenine type and CI-mass spectrometry gave a $[M + H]^+$ peak at *m/z* 316. ^1H and ^{13}C NMR spectra clearly showed the similarity of **1** with **4**, compound **1** exhibiting an additional *O*-methyl group at δ55.2. A long-range correlation experiment with intervals optimized for coupling constants (COLOC) allowed the identification of the connectivities in **1** through ^nJ(CH) coupling. The assignment of all quaternary C-atoms in this alkaloid type was possible by application of the COLOC-NMR technique, in addition to the H–C COSY. The attachment of the *O*-methyl group at C-7 was confirmed by long-range coupling between H-7 and the methyl group at δ55.2; C-7, C-7a and C-9 showed couplings with H-8 (similarly: C-10/H-11, C-11a/H-11 and C-11b/H-11). In this way, the signals at δ146.7 (*s*, C-10) and 146.8 (*s*, C-9), as well as the signals at δ109.5 (*d*, C-11) and 107.3 (*d*, C-8) could be assigned by long-range couplings with H-8 (*s*, 6.67) and H-11 (*s*, 6.77). The configuration of the *O*-methyl group (δ55.2) was determined by a NOE experiment. When irradiating with the frequency of the protons of the *O*-methyl group, a significant NOE at H-5a was observed, as well as a positive NOE at H-7.

In the series of lycorenane-7-ol alkaloids, 7-alkyl derivatives have been isolated from plant material [3–6]. Kihara *et al.* [6] assumed the isolated *O*-methyllycorenine (**7**) to be an artefact derived from lycorenine during the extraction of *Lycoris radiata* with methanol. Therefore, we investigated this possibility when we had elucidated the structure of **1**. We could prove the existence of **1** in the akaloids obtained from a methanol extract, as well as from an ethanol plant extract by means of HPLC and GC-mass spectrometry. The alkaloids obtained from the ethanolic extract had no contact with methanol during the whole isolation procedure. Thus, *O*-methyloduline (**1**) is a native plant alkaloid in *N. pseudonarcissus.* subsp. *pseudonarcissus* cv. Carlton and not an artefact derived from oduline and the solvent, methanol.

The NMR spectra of *N*-demethylmasonine (**2**) showed many similarities to the alkaloid, masonine (**5**), which we had isolated from the alkaloid mixture previously. However, a *N*-methyl group was missing. CI-mass spectrometry revealed the $[M + H]^+$ peak at *m/z* 286, which did not appear in the EI-mass spectrum. This is very often observed for alkaloids of the lycorenine type. Assignments of structure were possible from 2D-NMR investigations (H–H COSY, H–C COSY), conducted for masonine (**5**).

*Author to whom correspondence should be addressed.





(1) $R_1, R_2 = -CH_2-$ $R_3 = CH_3$

(2) $R_1 = H$ $R_2, R_3 = -CH_2-$

(3) $R_1, R_2 = CH_3$ $R_3 = H$

(5) $R_1 = CH_3$ $R_2, R_3 = -CH_2-$

(4) $R_1, R_2 = -CH_2-$ $R_3 = H$

(6) $R_1 = CH_3$ $R_2, R_3 = CH_3$

(7) $R_1, R_2 = CH_3$ $R_3 = CH_3$

(8) $R_1 = H$ $R_2, R_3 = CH_3$

It is remarkable, that besides *N*-demethylhomolycorine (**8**) (Reg. Nr. 6872-38-4), no other *N*-demethylated alkaloids of the lycorenine type have been reported before [7]. This is especially surprising, considering the biosynthesis of this alkaloid type via norbelladine and norpluviine [8]. The products of ring-conversion of the lycorenine-type alkaloids have to be *N*-methylated to give the well-known homolycorine- and lycorenine-type alkaloids. Therefore, we presume *N*-demethylated alkaloids of the lycorenine-type to be present in many Amaryllidaceae, but only in very low amounts. From 12 kg of fresh plant material only 27 mg of *N*-demethylmasonine (**2**) could be isolated.

EXPERIMENTAL

General. All mps are uncorr. NMR were recorded at 400 MHz for ^1H or 100 MHz for ^{13}C, with TMS as int. standard, chemical shifts in ppm, *J* in Hz.

Plant material. Bulbs of *N. pseudonarcissus* subsp. *pseudonarcissus* cv. Carlton from a culture in Sint Maarten (The Netherlands) were collected in August 1992. The plant was identified by Prof. Dr V. Melzheimer (Faculty of Biology, University of Marburg) and a voucher specimen (No. 0992Kreh) is deposited at the Faculty of Pharmacy, University of Marburg.

Extraction and isolation. Fr. bulbs (12 kg) were crushed and extracted $\times 3$ by maceration with 10 l MeOH (room temp.). The extract obtained after evapn of the MeOH was dissolved in H_2O, acidified with 1% HCl (pH 1.5) and filtered. After removal of neutral material with *n*-hexane (4×500 ml), the acidic soln was extracted with CH_2Cl_2 (4×500 ml). The soln was made basic with a $NaHCO_3$–Na_2CO_3 buffer (pH 9) and extracted once more with CH_2Cl_2 (5×500 ml). The organic phase was washed with H_2O and evapd, affording 30.08 g of alkaloids (0.25% fr. wt). The obtained alkaloids (30 g) were fractionated in a single step by CC using TSK HW 40S (Merck) as stationary phase (380×180 mm, 30 ml min^{-1}, H_2O–MeOH (7:3); 530–650 min linear gradient to 100% MeOH). Monitoring by UV-detector afforded 8 frs. Fr. VII (405–490 min) was further purified using the same stationary phase with H_2O–MeOH (1:1) at 40 ml min^{-1} affording 4 frs. Fr. VII-4 (220–300 min) afforded, after prep. HPLC (250×25 mm, TLC-KG 60H, 15 μm (Merck), 15 ml min^{-1}, *n*-hexane–EtOH–*iso*-PrOH–Et_2NH (67:21:10:2)), the alkaloids *O*-methyloduline (**1**) (230 mg), oduline (**4**) (32 mg), masonine (**5**) (28 mg), haemanthamine (765 mg) and narwedine (54 mg). Fr. VIII (490–650 min) was further purified using the same HPLC system as described for fr. VII-4, affording oduline (**4**) (47 mg), *N*-demethylmasonine (**2**) (27 mg), vittatine (92 mg), *N*-demethylgalanthamine (250 mg), epi-*N*-demethyllycoramine (50 mg) and an alkaloid (308 mg) of the pluviine-type (still under investigation).

GC-MS. Analyses were carried out as described in detail in ref. [9].

Analytical HPLC. LiChrospher® 60 RP-select B, 5 μm, 250×4 mm (Merck). 1 ml min^{-1}; A-B (1:3); A: MeCN, B: 2 g sodium dodecyl sulphate, 2 l H_2O, 400 ml MeCN, 290 ml 0.05 M H_3PO_4.

O-Methyloduline (**1**). Mp (picrate) 164°. $[\alpha]_D^{25}$ + 148 (MeOH; *c* 0.13). UV λ_{max}^{EtOH} (log ε) nm: 208 (4.43), 226 (3.85), 291 (3.58), 336 (2.39). IR ν_{max}^{KBr} cm^{-1}: 3409, 2903, 2781, 1624, 1504, 1484, 1438, 1387, 1358, 1342, 1282, 1241, 1186, 1124, 1099, 1042, 953, 901. ^1H NMR (400 MHz, $CDCl_3$): δ6.77 (1H, *s*, H-11), 6.67 (1H, *s*, H-8), 5.83 (2H, *d*, ^2J = 20.0, $-OCH_2O-$), 5.39 (2H, *br s*, H-7, H-4), 4.17 (1H, *d*, ^3J = 5.5, H-5), 3.43 (3H, *s*, –OMe), 3.08 (1H, *dd*, *J* = 8.0, 4.4, H-2), 2.69 (1H, *br d*, $^3J_{11b/11c}$ = 8.7, H-11c), 2.53 (1H, *dm*, ^2J = 19.2, ^3J = 3.1, H-5), 2.33–2.43 (2H, *m*, H-3), 2.35 (1H, *dd*, ^3J = 10.9, 1.1, H-11b), 2.26 (1H, *dd*, ^2J = 19.1, ^3J = 2.6, H-5), 2.17 (1H, *dd*, ^2J = 18.5, $^3J_{2/3}$ = 9.35, H-2), 2.05 (3H, *s*, NMe). ^{13}C NMR (100 MHz, $CDCl_3$): δ146.8 (*s*, C-9), 146.7 (*s*, C-10), 140.3 (*s*, C-3a), 131.8 (*s*, C-7a), 126.9 (*s*, C-11a), 115.6 (*d*, C-4), 109.5 (*d*, C-11), 107.3 (*d*, C-8), 100.8 (*t*, $-OCH_2O-$), 98.2 (*d*,

C-7), 67.5 (*d*, C-11c), 66.4 (*d*, C-5a), 56.5 (*t*, C-2), 55.2 (*q*, OMe), 44.0 (*q*, NMe), 43.9 (*d*, C-11b), 31.3 (*t*, C-5), 27.9 (*t*, C-3). EIMS, 70 eV, *m/z* (rel. int.): 109 (100), 108 (13), 85 (12), 175 (8), 283 (7), 110 (7), 44 (5), 94 (4), 47 (4), 284 (4), 42 (3), 315 (0.11 $[M]^+$). HRMS (for $[M - OMe]^+$, $C_{17}H_{18}NO_3$): found: 284.1287, requires 284.1291. Found (picrate): C, 53.11; H, 4.53; N, 10.02; O, 32.16. $C_{24}H_{24}N_4O_{11}$ requires: C, 52.94; H, 4.41; N, 10.29; O, 32.36%.

Oduline (**4**). Mp 165° (168° lit. [10]). $[\alpha]_D^{25}$ + 149.4 (MeOH; *c* 3.0). UV λ_{max}^{MeOH} (log ε) nm: 206 (4.42), 236 (3.51), 288 (3.47). IR ν_{max}^{KBr} cm^{-1}: 3440, 2941, 2860, 1750, 1632, 1502, 1480, 1390, 12452, 1038, 1001, 938, 899. ^{1}H NMR (400 MHz, $CDCl_3$): δ6.90 (*s*, 1H, H-11), 6.85 (*s*, 1H, H-8), 5.99 (*s*, 1H, H-7), 5.97 (*d*, ^2J = 14.7, 2H, $-OCH_2O-$), 5.46 (*br d*, $^3J_{4/5}$ = 2.9, 1H, H-4), 4.35 (*d*, ^3J = 6.0, 1H, H-5a), 3.14 (*ddd*, *J* = 9.2, 6.3, 3.8, 1H, H-2), 2.72 (*br d*, $^3J_{11b/11c}$ = 9.5, 1H, H-11c), 2.62 (*dm*, ^2J = 19.3, 1H, H-5), 2.43–2.50 (*m*, 3H, 2 H-3, H-11b), 2.31 (*dm*, ^2J = 19.0, ^3J = 2.8, 1H, H-5), 2.25 (*dd*, *J* = 18.7, 9.5, 1H, H-2), 2.11 (*s*, 3H, NMe). ^{13}C NMR (400 MHz, $CDCl_3$): δ147.0 (*s*, C-9), 147.0 (*s*, C-10), 140.6 (*s*, C-3a), 132.0 (*s*, C-7a), 128.2 (*s*, C-11a), 115.7 (*d*, C-4), 109.8 (*d*, C-11), 107.4 (*d*, C-8), 101.0 (*t*, $-OCH_2O-$), 91.8 (*d*, C-7), 67.5 (*d*, C-11c), 66.7 (*d*, 5a), 56.7 (*t*, C-2), 44.34 (*q*, –NMe), 44.22 (*d*, C-11b), 31.7 (*t*, C-5), 28.1 (*t*, C-3). MS (CI, iso-butane) *m/z* (rel. int.): 109 (100), 69 (43), 284 (39), 67 (37), 302 (32) $[M + H]^+$, 283 (30), 71 (27), 266 (24), 110 (15). Found: C, 67.94; H, 6.43; N, 4.27; O, 21.36. Calc. for $C_{17}H_{19}NO_4$: C, 67.77; H, 6.31; N, 4.65; O, 21.27%.

Masonine (**5**). Mp (picrate): 235°. $[\alpha]_D^{25}$ + 55.6 ($CHCl_3$; *c* 0.5), (lit. + 140; *c* 0.2 $CHCl_3$). UV λ_{max}^{MeOH} (log ε) nm: 206 (4.16), 225 (3.98), 270 (3.39), 304 (3.34). IR ν_{max}^{KBr} cm^{-1}: 3240, 2925, 2855, 1719, 1680, 1616, 1505, 1484, 1387, 1298, 1255, 1201, 1124, 1035. ^{1}H NMR (400 MHz, $CDCl_3$): δ7.49 (*s*, 1H, H-8), 6.96 (*s*, 1H, H-11), 6.07 (*dd*, *J* = 4.7, 0.9, 2H, $-OCH_2O-$), 5.50 (*br d*, $^3J_{4/5}$ = 2.2, 1H, H-4), 4.75 (*m*, *J* = 2.2, 2.1, 1H, H-5a), 3.18 (*ddd*, *J* = 7.4, 6.3, 4.2, 1H, H-2), 2.72–2.76 (*m*, *J* = 5.2, 1.6, 2H, H-11b, H-11c), 2.58–2.62 (*m*, *J* = 2.9, 2H, 2H-5), 2.48–2.52 (*br m*, 2H, 2H-3), 2.27 (*dd*, ^2J = 18.7, $^3J_{2/3}$ = 9.2, 1H, H-2), 2.06 (*s*, 3H, NMe). ^{13}C NMR (400 MHz, $CDCl_3$): δ165.4 (*s*, C-7), 151.8 (*s*, C-9), 147.8 (*s*, C-10), 140.2 (*s*, C-3a), 139.7 (*s*, C-11a), 118.5 (*s*, C-7a), 115.6 (*d*, C-4), 109.7 (*d*, C-8), 108.6 (*d*, C-11), 102.0 (*t*, $-OCH_2O-$), 77.3 (*d*, C-5a), 66.8 (*d*, C-11c), 56.3 (*t*, C-2), 43.8 (*d*, C-11b), 43.5 (*q*, NMe), 31.1 (*t*, C-5), 27.9 (*t*, C-3). MS (CI, iso-butane) *m/z* (rel. int.): 300 (100) $[M + H]^+$, 109 (92), 69 (71), 191 (29), 74 (24), 110 (16). Found: C, 67.34; H, 5.62; N, 4.61; O, 22.43. Calc. for $C_{17}H_{17}NO_4$: C, 68.23; H, 5.69; N, 4.68; O, 21.40%.

N-*Demethylmasonine* (**2**). Mp (picrate) 229°. $[\alpha]_D^{25}$ + 9.1 ($CHCl_3$; *c* 0.4). UV λ_{max}^{MeOH} (log ε) nm: 205 (4.26), 226 (4.25), 250sh (3.88), 263 (3.81), 304 (3.26) (3.26). IR ν_{max}^{KBr} cm^{-1}: 3427, 2922, 1714, 1616, 1504, 1462, 1387, 1296, 1259, 1036, 935. ^{1}H NMR (400 MHz, $CDCl_3$): δ7.52 (*s*, 1H, H-8), 7.11 (*s*, 1H, H-11), 6.05 (*d*, ^2J = 5.1, 2H, $-OCH_2O-$), 5.64 (*m*, *J* < 1, 1H, H-4), 4.85 (*m*, *J* < 1, 1H, H-5a), 5.50 (*br d*, $^3J_{11c/11b}$ = 9.3, 1H, H-11c), 3.15 (*ddd*, *J* = 10.8, 6.8, 4.3, 1H, H-2), 2.89 (*dd*, *J* = 18.6, 8.6, 1H, H-2), 2.66 (*d*, $^3J_{11c/11b}$ = 9.7, 1H, H-11b), 2.62 (*m*, 2H, 2H-5), 2.52 (*m*, *J* = 7.6, 2H, H-3). ^{13}C NMR (400 MHz, $CDCl_3$): δ165.1 (*s*, C-7), 152.2 (*s*, C-9), 147.8 (*s*, C-10), 139.4 (*s*, C-3a), 138.7 (*s*, C-11a), 118.0 (*s*, C-7a), 115.1 (*d*, C-4), 109.9 (*d*, C-8), 107.4 (*d*, C-11), 102.0 (*t*, $-OCH_2O-$), 75.6 (*d*, C-5a), 59.1 (*d*, C-11c), 44.1 (*t*, C-2), 43.0 (*d*, C-11b), 31.2 (*t*, C-5), 29.6 (*t*, C-3), MS (CI, iso-butane *m/z* (rel. int.): 69 (100), 71 (87), 286 (14) $[M + H]^+$, 123 (7), 191 (4). Found: C, 66.98; H, 5.19; N, 4.96; O, 22.87. Calc. for $C_{16}H_{15}NO_4$: C, 67.37; H, 5.26; N, 4.91; O, 22.46%.

REFERENCES

1. Jefferson-Brown, M. (1991) *Narcissus*, p. 122. B. T. Batsford Ltd, London.
2. Mondon, A. and Krohn, K. (1975) *Chem. Ber.* **108**, 445.
3. Numata, A., Takemura, T., Ohbayashi, H. Katsuno, T., Yamamoto, K., Sato, K. and Kobayashi, S. (1983) *Chem. Pharm. Bull.* **31**, 2146.
4. Via, J., Arriortua, M. I., Ochando, L. E., Reventos, M. M., Amigo, J. M. and Bastida, J. (1989) *Acta Cryst.* **C45**, 2020.
5. Codina, C., Bastida, J., Viladomat, F., Fernández, J. M., Bergoñón, S., Rubriralta, M. and Quirion, J. C. (1993) *Phytochemistry* **35**, 1354.
6. Kihara, M., Konishi, K., Xu, L. and Kobayashi, S., (1991) *Chem. Pharm. Bull.* **39**, 1849.
7. Tsakadze, D. M., Kiparenko, T. N., Tsitsishvili, N. S. and Abdusamatov, N. S. (1969) *Soobshch. Akad. Nauk Gruz. SSR* **56**, 305.
8. Mothes, K., Schütte, H. R. and Luckner, M. (1985) *Biochemistry of Alkaloids*, p. 241. VEB Deutscher Verlag der Wissenschaften, Berlin.
9. Kreh, M., Matusch, R. and Witte, L. *Phytochemistry* **38**, 773..
10. Boit, H. G., Stender, W. and Beitner, A. (1957) *Chem. Ber.* **90**, 725.