Pergamon

0031-9422(95)00465-3

Phytochemistry, Vol. 40, No. 4, pp. 1303–1306, 1995

0031-9422/95 $9.50 + 0.00

ACETYLATED ALKALOIDS FROM *NARCISSUS PSEUDONARCISSUS**

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(*Received* 31 *October* 1994)

Key Word Index—*Narcissus pseudonarcissus*; Amaryllidaceae; daffodil; alkaloids; GC–MS coupling; norpluviine; 10-norpluviine; 1-*O*-acetyl-10-norpluviine; 1,10-diacetyl-10-norpluviine; *O*-acetylgalanthamine.

Abstract—Three new alkaloids, 10-norpluviine, 1-*O*-acetyl-10-norpluviine and 1,10-diacetyl-10-norpluviine, have been found in bulbs of *Narcissus pseudonarcissus*. The known *O*-acetylgalanthamine is reported for the first time as a natural product. 1,10-Diacetyl-10-norpluviine and *O*-acetylgalanthamine were identified by means of gas chromatography–mass spectrometry and were prepared from 10-norpluviine and galanthamine. By COLOC and NOE experiments it was shown that 10-norpluviine is not identical with norpluviine, which should be named correctly as 9-norpluviine.

INTRODUCTION

Recently, we reported the isolation of 15 alkaloids from *Narcissus pseudonarcissus* L. ssp. *pseudonarcissus* cv Carlton [1] and an alkaloid of the pluviine type. This alkaloid was identified as 10-norpluviine (**1**) by NMR spectroscopy (COLOC and NOE experiments). Continuing our GC–mass spectrometry (MS) studies on this plant [2], we report now the isolation of the acetylated alkaloids 1-*O*-acetyl-10-norpluviine (**2**), 1,10-diacetyl-10-norpluviine (**3**) and *O*-acetyl-galanthamine (**4**) from this daffodil.

RESULTS AND DISCUSSION

Compound **1** was isolated as described in ref. [1]. The proton NMR spectrum of **1** reveals two *para*-oriented aromatic protons (6.82, *s*, 6.59, *s*), an aromatic methoxyl group (3.89, *s*), benzyl protons (3.93 and 3.31, each *d*, $^2J = 14.0$), an olefinic proton (5.54, *d*, $^3J_{2/3} = 7.0$), but no *N*-methyl group. These data suggest a lycorine-type skeleton. However, the ^{13}C-DEPT-NMR spectrum reveals a methylene carbon (33.5, *t*) instead of the C-2 methine carbon (*ca* 72 ppm, *d*) for the lycorine type. So the alkaloid was identified as a demethylated derivative of pluviine (**5**).

A pluviine derivative with only a single aromatic methoxy group was isolated by Uyeo and Yanaihara from *Lycoris radiata* [3]. They named this alkaloid norpluviine (**6**). The position of the methoxyl group in **6** was established by the conversion of **6** into a synthetic available phenanthridone. However, the physical constants of the alkaloid **1**, isolated from *N.* '*Carlton*' were not in agreement with that of **6** [3, 4].

To avoid the degradation of **1** in a similar manner, we decided to apply the COLOC-NMR technique [correlation via long range couplings; identification of connectivities through ^nJ(CH) coupling] to this alkaloid. The COLOC-NMR spectrum shows clearly the ^4J long range couplings between the protons of the methyl group and the quaternary C-9 as well as between C-9 and H-8. So the novel alkaloid **1** is an isomer of the known norpluviine (**6**), which should be named correctly 9-norpluviine.

The *trans*-linkage of the rings B and C in lycorine-type alkaloids has been well known for many years. However, for the alkaloid fortucine a *cis*-linkage was described, deduced from the coupling constants between H-11b and H-11c (6.0 Hz) [5, 6]. To obtain further evidence for structure **1**, we decided to perform a series of NOE experiments. Table 1 shows the results, which prove the *trans*-linkage in **1** unambiguously.

The EI mass spectrum of **2** was similar to that of **1**. In addition to an intense $[M]^+$ peak at *m/z* 315 we found characteristic fragment ions at *m/z* 255 $[M - 60]^+$, 254 $[M - 60 - H]^+$ and 43 (H_3CCO^+). This fragmentation

* Dedicated to Professor Dr F. Eiden, Munich, F.R.G., on the occasion of his 70th birthday.

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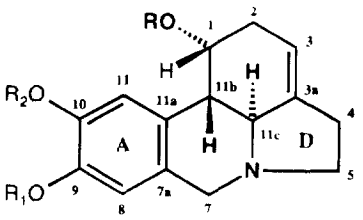
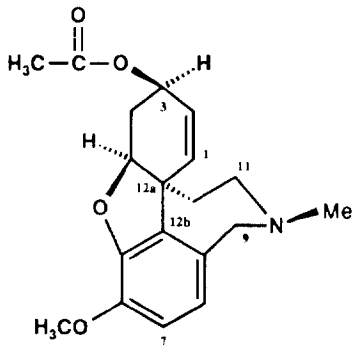


	R	R_1	R_2
(1)	H	Me	H
(2)	Ac	Me	H
(3)	Ac	Me	Ac
(5)	H	Me	Me
(6)	H	H	Me



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pattern is only possible if an acetyl group is attached at C-1 (Scheme 1). The structure was proved by synthesis of **2** from **1**. The reaction was carried out in a routine procedure with acetylchloride as reagent, but without the use of pyridine. By this way we obtained **2** in 70% yield besides the diacetate (**3**) and unreacted **1**.

By means of GC–mass spectrometry coupling we examined an *n*-heptane extract, obtained directly from bulbs of our daffodil after basification with NH_3. Besides **1**, **2**, the alkaloids already reported in ref. [2], several fatty acids and their methyl esters, we found an alkaloid, whose mass spectral fragmentation pattern suggested a diacetyl derivative of **1**. 1,10-Diacetyl-10-norpluviine (**3**) was prepared from **1** by reaction with acetic anhydride–pyridine. Its mass spectrum and GC retention index (R_I) were identical with that of the alkaloid from the *n*-heptane extract (Table 2).

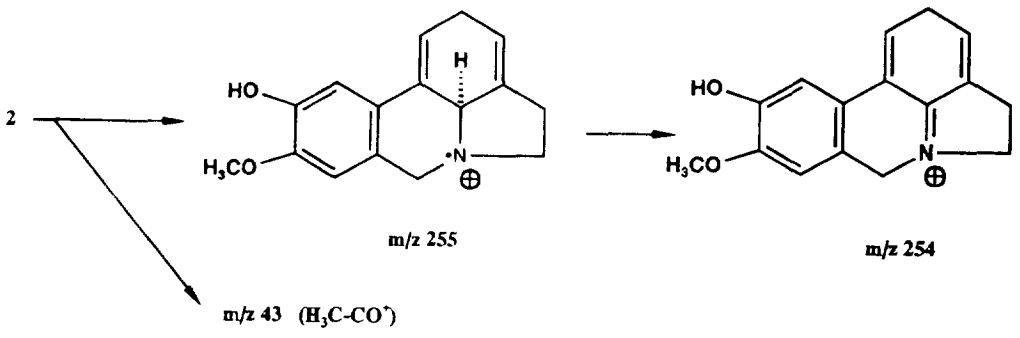
In a similar manner the alkaloid **4** was identified in the *n*-heptane extract. Compound **4** was synthesized in the past for structural studies on galanthamine-type alkaloids [7, 8] and pharmacological studies [9], but to the best of our knowledge it is reported here for the first time as a natural product. This alkaloid was found only in trace amounts by GC–MS, so we did not try to isolate it, but synthesized **4** as described in ref. [9]. Spectroscopic data for the product were in agreement with those of ref. [9]. The mass spectra and GC retention index of the product were identical with those of the alkaloid found during GC–MS (Table 2).

EXPERIMENTAL

General, plant material, extraction and isolation. See ref. [1]. Compound **1** was obtained from fr. VIII (490–650 min) as described. Compound **2** was obtained

Table 1. NOE effects observed for 10-norpluviine (**1**)

Irradiation at	NOE at H-1	NOE at H-11	NOE at H-11b	NOE at H-11c
H-1		+	+	–
H-11	+		+	–
H-11b	(+)	+		–
H-11c	–	–	–	



Scheme 1. Fragmentation of 1-*O*-acetyl-10-norpluviine (**2**) during GC–MS.

Table 2. Mass spectral data and retention indices obtained during GC–MS

Alkaloid	Characteristic ions *m z* (rel.int.)	RI on DB-1 GC column
1	273 (78, $[M]^+$), 272 (100), 257 (8), 256 (16), 244 (27), 228 (42), 216 (14), 162 (12), 147 (13), 95 (15)	2440
2	315 (48, $[M]^+$), 314 (53), 256 (28), 255 (37), 254 (100), 242 (15), 228 (29), 43 (32)	2410
3	329 (27, $[M]^+$), 328 (22), 270 (100), 216 (36), 165 (17), 115 (13), 43 (27)	2456
4	357 (50, $[M]^+$), 356 (53), 315 (24), 314 (37), 297 (43), 296 (53), 254 (100), 43 (55)	2545

from fr. VI (373–405 min) by CC on TSK HW 40S (Merck) as stationary phase (380 × 180 mm), 40 ml min^{-1} 0.2% HCO_2H–MeOH (1:1) and further purification by prep. HPLC on LiChrosorb, 250 × 25 mm, 39 ml min^{-1}, *n*-hexane–*iso*PrOH–CH_2Cl_2–Et_2NH (70:30:5:0.02). GC–MS: see ref. [2].

10-Norpluviine (**1**). Mp 148°, decomp. > 136°. $[\alpha]_D^{25}$ = + 61 (MeOH; *c* = 2.4). UV, nm: λ_{max}^{MeOH} (log ε) 205 (4.43), 228 (3.72), 286 (3.47). ^{1}H NMR (400 MHz, $CDCl_3$), δ:6.82 (1H, *s*, H-11), 6.59 (1H, *s*, H-8), 5.54 (1H, *brd*, $^3J_{2,3}$ = 7.0, H-3), 4.26 (1H, *dd*, $^3J_{1,2}$ = 5.2, $^3J_{1,11b}$ = 2.6, H-1), 3.93 (1H, *d*, ^2J = 14.0, H-7), 3.89 (3H, *s*, OMe), 3.32 (1H, *m*, H-5), 3.31 (1H, *d*, ^2J = 14.0, H-7), 2.98 (1H, *dd*, $^3J_{11b,11c}$ = 5.8, $^3J_{1,11b}$ = 2.3, H-11b), 2.70 (1H, *brd*, $^3J_{11c,11b}$ = 5.2, H-11c), 2.46–2.56 (3H, *m*, 2 × H-4, H-2), 2.32–2.41 (2H, *m*, H-5, H-2). ^{13}C NMR (100 MHz, $CDCl_3$), δ:146.0 (*s*, C-9), 144.4 (*s*, C-10), 139.6 (*s*, C-3a), 128.8 (*s*, C-11a), 126.0 (*s*, C-7a), 116.2 (*d*, C-3), 112.4 (*d*, C-8), 110.2 (*d*, C-11), 69.8 (*d*, C-1), 59.5 (*d*, C-11c), 55.9 (*q*, OMe), 55.3 (*t*, C-7), 52.1 (*t*, C-5), 41.6 (*d*, C-11b), 33.5 (*t*, C-2), 27.3 (*t*, C-4). EIMS, 70 eV, *m z* (rel. int.): 272 (100), 273 (78, $[M]^+$), 252 (26), 228 (25), 244 (20), 58 (19), 229 (14), 253 (14). HRMS (for $[M]^+$, $C_{16}H_{19}NO_3$): found: 273.1365, requires 273.1350.

1-*O*-*Acetyl*-10-*norpluviine* (**2**). Mp 203°, decomp. > 180°. $[\alpha]_D^{25}$ = − 38 (MeOH; *c* = 1.0). UV, nm: λ_{max} 224, 285 (in *n*-hexane–EtOH–*iso*PrOH–Et_2NH; 70:25:5:0.01). ^{1}H NMR (400 MHz, $CDCl_3$), δ:7.08 (1H, *s*, H-8), 6.64 (1H, *s*, H-11), 5.54 (1H, *dd*, $^3J_{1,11b}$ = 5.7, $^3J_{1,2}$ = 9.8, H-1), 5.39 (1H, *brs*, H-3), 3.86 (3H, *s*, OMe), 3.59 (1H, *brd*, ^2J = 14.3, H-7), 3.54 (1H, *brd*, ^2J = 14.3, H-7), 3.33 (1H, *dd*, ^3J = 7.3, [5.0], H-11b), 2.79 (2H, *brm*, 2 H-5), 2.28–2.50 (5H, *m*, *J* = 7.0, 2.4, H-11c, 2 H-4, 2 H-2), 1.98 (3H, *s*, $H_3C_2O_2$). ^{13}C NMR (100 MHz, $CDCl_3$), δ:170.6 (*s*, C=O), 145.4 (*s*, C-9), 144.0 (*s*, C-10), 139.2 (*s*, C-3a), 129.2 (*s*, C-11a), 125.0 (*s*, C-7a), 115.0 (*d*, C-3), 113.4 (*d*, C-8), 110.2 (*d*, C-11), 70.3 (*brd*, C-1), 60.4 (*d*, C-11c), 56.0 (*q*, OMe), 52.4 (*t*, C-5), 51.9 (*brt*, C-7), 38.0 (*brd*, C-11b), 28.8 (*t*, C-2), 28.4 (*t*, C-4), 21.4 (*q*, H_3CCOO). EIMS, 70 eV, *m z* (rel. int.): 254 (100), 314 (73, $[M-H]^+$), 315 (65, $[M]^+$), 255 (43, $[M-60]^+$), 256 (34, $[M-H_3CCOO^\cdot]^+$), 43 (31, H_3CCO^+), 286 (30), 228 (29), 252 (28), 287 (24), 226 (23). HRMS (for $[M]^+$, $C_{18}H_{21}NO_4$): found: 315.1504, requires 315.1471.

1,10-*Diacetyl*-10-*norpluviine* (**3**). Mp 147° (EtOH). $[\alpha]_D^{25}$ = + 8.3 (MeOH; *c* = 1.1). UV, nm: λ_{max} 203, 279 (in *n*-hexane–EtOH–*iso*PrOH–Et_2NH; 70:25:5:0.01). ^{1}H NMR (400 MHz, $CDCl_3$), δ:7.14 (1H, *brs*, H-8), 6.71 (1H, *s*, H-11), 5.51 (1H, *brm*, H-1), 5.32 (1H, *brt*, $^3J_{2,3}$ = 3.2, H-3), 3.74 (3H, *s*, OMe), 3.48–3.60 (2H, *brs*, 2 × H-7), 3.27 (1H, *dd*, ^3J = 7.2, 4.1, H-11b), 2.58–2.81 (2H, *brm*, 2 × H-5), 2.36 (3H, *m*, $^2J_{2/3}$ = 3.3, 2 × H-2, H-4), 2.23 (3H, *s*, $H_3C_2O_2$ at C-10), 2.17–2.32 (2H, *m*, H-4, H-11c), 1.92 (3H, *brs*, H_3CCOO at C-1). ^{13}C NMR (100 MHz, $CDCl_3$), δ:170.6 (*s*, C = O at C-1), 169.2 (*s*, C=O at C-10), 149.4 (*s*, C-9), 139.4 (*s*, C-3a), 137.5 (*s*, C-10), 132.5 (*s*, C-11a), 129.2 (*s*, C-7a), 121.2 (*d*, C-11), 114.6 (*brd*, C-3), 111.5 (*d*, C-8), 69.9 (*brd*, C-1), 60.2 (*d*, C-11c), 55.9 (*q*, OMe), 52.3 (*t*, C-5), 51.8 (*brt*, C-7), 38.2 (*brd*, C-11b), 28.8 (*brt*, C-2), 28.6 (*brt*, C-4), 21.3 (*q*, $H_3C_2O_2$ at C-10), 20.6 (*q*, $H_3C_2O_2$ at C-1). EIMS, 70 eV, *m z* (rel. int.): 254 (100), 252 (90), 356 (61, $[M-H]^+$), 43 (57, $H_3C_2O^+$), 296 (55), 357 (50, $[M]^+$), 255 (45), 314 (43), 297 (42), 253 (32), 226 (30), 256 (26), 315 (25, $[\mathbf{2}]^+$). HRMS (for $[M]^+$, $C_{20}H_{23}NO_5$): found: 357.1564, requires 357.1576.

O-*Acetylgalanthamine* (**4**). Mp.: 129° (EtOH). $[\alpha]_D^{25}$ = − 61 (MeOH; *c* = 1.3). UV, nm: λ_{max}^{MeOH} (log ε) = 209 (3.51), 230 (2.85), 287 (2.42) nm (MeOH). ^{1}H NMR (400 MHz, $CDCl_3$), δ:6.68 (1H, *d*, $^3J_{7/8}$ = 8.2, H-7), 6.61 (1H, *d*, $^3J_{7/8}$ = 8.2, H-8), 6.28 (1H, *d*, $^3J_{1,2}$ = 10.4, H-1), 5.92 (1H, *dd*, $^3J_{1/2}$ = 10.3, $^3J_{2/3}$ = 4.9, H-2), 5.34 (1H, *dd*, $^3J_{2/3}$ = 5.2, $^3J_{3/4} \approx 1$, H-3), 4.58 (1H, *m*, *J* < 1, H-4a), 4.22 (1H, *d*, ^2J = 15.2, H-9), 3.85 (3H, *s*, OMe), 3.77 (1H, *d*, ^2J = 15.2, H-9), 3.40 (1H, *brt*, ^2J = 13.1, H-11), 3.14 (1H, *brd*, ^3J = 14.5, H-11), 2.69 (1H, *ddd*, ^2J = 16.4, ^3J = 1.3, 1.5 H-4), 2.45 (3H, *s*, NMe), 2.18 (1H, *dd*, ^2J = 13.7, $^3J_{11/12}$ = 2.8, H-12), 2.10 (1H, *ddd*, ^2J = 16.7, ^3J = 3.3, 2.2, H-4), 2.04 (3H, *s*, $H_3C_2O_2$), 1.65 (1H, *dd*, ^2J = 13.9, ^3J < 1, H-12). ^{13}C NMR (100 MHz, $CDCl_3$), δ:170.9 (*s*, C=O), 146.7 (*s*, C-6), 144.4 (*s*, C-5a), 131.9 (*s*, C-12b), 130.2 (*d*, C-8), 127.2 (*s*, C-8a), 123.3 (*d*, C-2), 121.8 (*d*, C-1), 111.7 (*d*, C-7), 86.2 (*d*, C-4a), 63.2 (*d*, C-3), 59.9 (*t*, C-9), 56.0 (*q*, OMe), 53.4 (*t*, C-11), 47.8 (*s*, C-12a), 40.9 (*q*, NMe), 33.7 (*t*, C-12), 27.7 (*t*, C-4), 21.4 (*q*, $H_3C_2O_2$). EIMS, 70 eV, *m/z* (rel. int.): 270 (100, $[M-59]^+$), 329 (46, $[M]^+$), 328 (37, $[M-H]^+$), 216 (25), 43 (24, $H_3C_2O^+$), 271 (19), 226 (13), 42 (11), 211

(12), 165 (11), 213 (11), 115 (11). HRMS (for $[M]^+$, $C_{19}H_{23}NO_4$): found: 329.1622, requires 329.1627.

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