Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Assiut, Egypt

Two new chlorinated amides from *Nicotiana glauca* R. Graham

E. Y. Backheet and H. M. Sayed

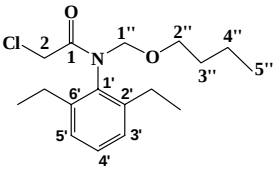
Two new chlorinated amides, *N*-(2′,6′-diethyl phenyl)-2-chloroacetamide (**1**) and *N*-(butyloxymethyl)-*N*-(2′,6′-diethyl phenyl)-2-chloroacetamide (**2**) were isolated for the first time from the ethanolic extract of the leaves of *Nicotiana glauca* R. Graham in addition to triacontanol (**3**), scopoletin (**4**) and stigmasterol-3-β-O-D-gluco-pyranoside (**5**). The structures of the isolated compounds were elucidated by spectroscopic analysis (1D, 2D NMR, EIMS, HR-EIMS, IR and UV).

1. Introduction

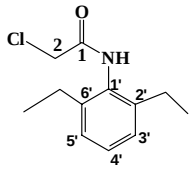
Nicotiana glauca R. Graham (Solanaceae) is a plant common in Egypt where it grows wildly. The plant is known locally by the Arabic name "Musseyss" [1]. It is occasionally used as an ornamental and in folk medicine for the treatment of burns and inflammatory diseases [2]. Previous work performed on this plant reported the occurrence of the alkaloid anabasine [1], rutin [3], an amide of the ethylene diamine type [4], a sesquiterpenoid (glutinosone) [5], and coumarins (scopoletin and its glucoside scopolin) [6]. Saitoh et al. reported the analysis of four alkaloids in the leaves and roots of 60 *Nicotiana* species and found that nicotine was the dominant alkaloid in the leaves of 33 species, nornicotine of 24 species, anabasine of 2 species (*N. glauca and N. debeneyi*) and anatabine of *N. otophora* only [7]. Snook et al. reported the identification of the flavonoids quercetin-3-rutinoside and 3-sophoroside, kaempferol-3-sophoroside and -3-rhamnosyl galactoside [8]. In the present work, we report the isolation and identification of two new chlorinated amides.

2. Investigations, results and discussion

The 70% ethanol extract of the leaves of *Nicotiana glauca* R. was fractionated by partitioning between chloroform and ethyl acetate. The chloroformic fraction yielded compounds **1**, **2**, and **3** by chromatography over silica gel column.



Compound 1



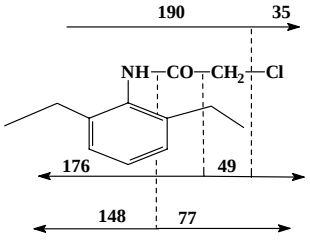
Compound 2

DEPT ^{13}C NMR spectra of compound **2** showed 12 carbon peaks, including two equivalent aromatic methine carbons at δ 126.31 and one at δ 128.28, two equivalent non-hydrogenated carbons at δ 131.44 and one at δ 141.21, two equivalent methyl carbons at δ 14.24, two equivalent methylene carbons at δ 24.60, one methylene carbon (CH_2Cl) at δ 42.59, and one non-hydrogenated carbon signal at δ 165.01, which could be assigned to an amide-like carbon (CO−N). The occurrence of an amide-like carbon was also supported by IR data indicating one strong characteristic carbonyl band at 1683 cm^{-1}.

The ^1H NMR ($CDCl_3$, 400 MHz) spectrum of **2** showed the presence of sixteen protons in two main groups of signals: four protons in the aromatic field (δ 7.14–7.97), ten protons assigned for two ethyl groups (quartet-triplet) attached to a benzene ring at position 2′ and 6′ (14.24 and 24.60 in ^{13}C NMR spectrum), and two protons in the high field area δ 4.20 (42.59 in ^{13}C NMR spectrum). Among signals in the aromatic field, a broad signal at δ 7.97, exchangeable with D_2O, was determined as a NH group since there was no signal for a carbon atom bearing a hydroxyl group in the ^{13}C NMR spectrum. The NH group was further confirmed by the presence of a strong absorption band at 3425 cm^{-1} in the IR. The other two aromatic signals centered at 7.14 (duplet) and 7.26 (triplet) were assigned to three protons of tri-substituted benzene (A_2X system) with a clear ortho coupling of 7.80 Hz. The singlet signal at δ 4.20 (42.59 in ^{13}C NMR spectrum) was determined as CH_2Cl. The presence of a chlorine atom in the structure was evidenced from the fragmentation pattern in EIMS which showed a loss of chlorine atom $[M\text{-}35]^+$, CH_2Cl $[M\text{-}49]^+$ to give the base peak [176]. The EIMS of compound **2** showed an ion at m/z 225, which was then confirmed to be the molecular ion peak. The presence of a chlorine atom was further confirmed by the isotope pattern $[M + 2]^+$ 227 due to the ^{37}Cl isotope and by elemental analysis for chlorine [calculated 15.55%, found 15.4%]. The molecular formula of $C_{12}H_{16}NOCl$ was confirmed by the high-resolution mass spectrometry and the elemental analysis for chlorine. The Scheme shows various fragmentations, which support the sequence of $ClCH_2-CO-NH-$benzene for compound **2**.

The ^1H- and ^{13}C NMR spectra were assigned with the aid of DEPT and CH−COSY techniques. ^{13}C NMR data are quite convenient as that calculated for tri-substituted benzene and other fragments [9]. Compound **1** data when compared with compound **2** revealed the presence of additional signals in the ^1H NMR spectrum at δ 0.75 (t, CH_3), 1.21 (sextet, CH_2), 1.40 (pentet, CH_2), 3.53 (triplet, CH_2) and 4.85 (singlet, CH_2) corresponding for butyl oxy methyl group. The corresponding signals in ^{13}C NMR were at δ 13.34, 18.66, 31.33, 69.85, 78.79. Also the broad signal corresponding for the NH proton in compound **2** was not found in compound **1**. The presence of

Scheme



butyloxy methyl was established from the coupling pattern as well as DEPT and CH-COSY techniques.

Also, as in compound **2**, the presence of a chlorine atom in the structure of compound **1** was evidenced from the fragmentation pattern in EIMS which showed a loss of chlorine atom $[M\text{-}35]^+$, and CH_2Cl $[M\text{-}49]^+$ respectively. The presence of a base peak at the same mass unit (m/z = 176) indicated the same structure as that of compound **2**. The EIMS of compound **1** showed an ion at m/z 311, which was confirmed to be the molecular ion peak. The presence of a chlorine atom was further confirmed by the isotope pattern $[M + 2]^+$ 313 due to the ^{37}Cl isotope and by elemental analysis for chlorine [calculated 11.25%, found 11.05%]. The molecular formula of $C_{17}H_{26}NO_2Cl$ was confirmed by high-resolution mass spectrometry.

The isolated amides **1** and **2** could not be traced in the literature. Such chlorinated compounds could not be considered as artifacts. The manipulation techniques did not involve halogen such as Cl_2 in any of the separation steps. If chlorine is added in a chemical reaction to *N*-(2′,6′-diethylphenyl)-2-actamide, the product will be *N*-(2′,6′-diethyl-4-chlorophenyl)-2-actamide, as halogenation will occur in the activated benzene ring and not at the side chain [10] .

The chloroacetyl entity has to be formed biogenetically by some means utilizing the high contents of acetic acid [11,12] and chlorine in *Nicotiana* species [13]. The presence of active methylene function ($COCH_2Cl$) in compounds **1** and **2** was supported by their reaction with sodium nitroprusside in the presence of sodium hydroxide to give a red colour [14].

It is noteworthy that, fatty acid amides are widespread in nature [15], they were incorporated into some lipid molecules such as ceramides, glyco-sphingolipids, *N*-acetylated lipids and bacterial lipoproteins [16]. Fatty acid amides are lipid bioregulators formed from long chain saturated and unsaturated fatty acids via amidation by the corresponding amines.

In the MS of compound **3**, m/z = 438 assigned the molecular formula $C_{30}H_{62}O$. It showed a peak at m/z = 420 $[M-H_2O]^+$ and a homologous series of peaks resulting from cleavage at C−C bonds successively removed from the molecule (hydrocarbon pattern). It showed IR absorption band (KBr) at 3420 cm^{-1} suggesting the presence of −OH group(s). Its ^1H NMR spectrum (400 MHz, $CDCl_3$) displayed signals at δ 0.86 (3 H, t, J = 6.0 Hz, a terminal CH_3), 1.25 (54 H, br s, 27 CH_2), 1.58 (2 H, m, CH_2 attached to CH_2OH), 3.64 (2 H, t, CH_2OH) indicating a long chain aliphatic alcohol. Its ^{13}C NMR spectrum displayed signals at 14.11 (C-1), 22.69 (C-2), 25.73 (C-3), 29.36 (C-26), 29.43 (C-27), 29.69 (C4-25), 31.92 (C-28), 32.80 (C-29) and 63.10 (C-30). The assignments of ^{13}C NMR chemical shifts were in good agreement with calculated values [17]. The spectral data of the alcohol **3** confirm the structure as triacontanol (myricyl alcohol).

The ethyl acetate fraction afforded compounds **4** and **5**. The comparison of the spectral data of compounds **4** and **5** with literature data approved that these compounds are scopoletin [18] and stigmasterol-3-*O*-β-D-glucoside [19].

The two new isolated chloroamides have not been previously reported and are separated here for the first time.

3. Experimental

3.1. General

UV spectra were run in ethanol using a Schimadzu UV/VIS-1602, Tokyo, Japan, and an Uvidec-320, Tokyo, Japan spectrophotometer with 1 cm quartz cuvettes. IR spectra were taken in KBr using IR-470 Schimadzu, Japan. 1D and 2D ^1H- and ^{13}C NMR spectra were run in $CDCl_3$ and C_5D_5N at 400 MHz by JEOL TNM-LA400, FT NMR system, Japan, using TMS as internal standard. EI-MS and HR-EIMS spectra were recorded by JEOL, JMS 600 H, Japan. Column chromatography using silica gel (E. Merck, Germany). TLC using silica gel G_{60} F_{254} activated layers (E. Merck, Germany) and precoated aluminium sheets (E. Merck, Germany).

The following solvent systems were used for TLC screening: n-Hexane−acetone (94 : 6) (I), n-Hexane−acetone (90 : 10) (II), Chloroform−methanol (90 : 10) (III).

3.2. Plant material

The leaves of *Nicotiana glauca* R. were collected during the flowering season 2000 from the plants cultivated in the experimental station, Faculty of Pharmacy, Assiut University. The collected material was air-dried, reduced to powder No. 40 and kept for extraction.

3.3. Processing of the plant material

The air-dried powdered leaves (1850 g) of *Nicotiana glauca* R. were exhaustively extracted with ethanol 70%, concentrated under reduced pressure and the concentrate (150 g) was then diluted with distilled water and subjected to solvent fractionation using chloroform (6 × 500 ml) and ethyl acetate (5 × 500 ml). The obtained fractions were separately concentrated and examined by TLC for different constituents.

3.4. Separation of the compounds

The chloroform soluble fraction (30 g) was further fractionated on a silica gel column (900 g, 7 cm (ID) × 210 cm (L). Elution was started with n-hexane followed by different proportions of n-hexane/chloroform. Fractions, 150 ml each, were collected, concentrated and screened by TLC (silica gel G, solvent system I). Similar fractions were combined, concentrated and subjected to purification and crystallization. The fractions eluted with n-hexane/chloroform (8 : 2) were subjected to silica gel preparative plates (solvent system I) to afford compounds **1** and **2**. Fractions eluted with chloroform were subjected to purification and crystallization to afford compound **3**.

The ethyl acetate soluble fraction (40 g) was further fractionated on silica gel column (1200 g, 7 cm (ID) × 210 cm (L). Elution was started with chloroform followed by different proportions of chloroform/methanol. Fractions of 250 ml were collected, concentrated and screened by TLC. Fractions eluted with chloroform/methanol (85 : 15) were subjected to silica gel preparative plates (solvent system III) to afford compound **4**. Fractions eluted with chloroform/methanol (8 : 2) were subjected to purification and crystallization to yield compound **5**.

3.5. Compound 1

Pale yellow oily residue (500 mg), $R_f = 0.71$ (system I), UV λ_{max}: (EtOH): 236, 265, and 273 nm. IR, υ_{max} (KBr), cm^{-1}: 3265, 1655, 1525, 1466, 1371, 1325, 1250, 1138, 983, 867,798, 720 and 650. ^1H NMR (400 MHz, $CDCl_3$) at δ: 0.75 (3 H, t, J = 7.32 Hz, 5″-CH_3), 1.10 (6 H, t, J = 7.60 Hz, 2 $\underline{CH_3}CH_2$), 1.21 (2 H, sex, J = 7.32 Hz, 4″-CH_2), 1.40 (2 H, pentet, J = 6.60 Hz, 3″-CH_2), 2.41 (2 H, q, J = 7.56 Hz, $\underline{CH_2}CH_3$), 2.46 (2 H, q, J = 7.56 Hz, $\underline{CH_2}CH_3$), 3.53 (2 H, t, J = 6.56 Hz, 2″−CH_2), 3.55 (2 H, s, CH_2Cl), 4.85 (2 H, s, 1″-$\underline{CH_2}$), 7.06 (2 H, d, J = 7.56 Hz, H-3′,5′), 7.17 (1 H, t, J = 7.56 Hz, H-4′). ^{13}C NMR (100 MHz, $CDCl_3$) at δ: 13.34 (5″-CH_3), 13.88 (2 $\underline{CH_3}CH_2$), 18.66 (4″-CH_2), 23.20 ($\underline{CH_2}CH_3$), 31.33 (3″-CH_2), 41.57 CH_2Cl), 69.85 2″-CH_2), 78.79 1″-CH_2), 126.45 (C-3′,5′), 128.77 (C-4′), 137.00 (C-1′), 141.18 (C-2′,6′), 167.23 (CO). EI^+-MS: $[M]^+$ m/z: 311 (9.8%), calculated for $C_{17}H_{26}NO_2Cl$. Other fragments at m/z = 276 (8.2%), 262 (2.3%), 237 (33.6%), 238 (24.5%), 224 (10.8%), 225 (10.4%), 187 (38.2%), 189 (10.7%) and 176 (100%). HR EI^+-MS: calculated: 311.1654, found: 311.1331.

3.6. Compound 2

White crystalline prisms (250 mg), $R_f = 0.45$ (system I), m.p. 124–126 °C, UV, λ_{max} (EtOH): 238, 265 and 270 nm. IR, υ_{max} (KBr), cm^{-1}: 3550, 3425, 2965, 1683, 1456, 1369, 1313, 1250, 1079, 809, and 636. ^1H NMR (400 MHz, $CDCl_3$) at δ: 1.20 (3 H, t, J = 7.56 Hz, $\underline{CH_3}CH_2$), 2.57 (2 H, q, J = 7.56 Hz, $\underline{CH_2}CH_3$), 4.20 (2 H, s, $\underline{CH_2}Cl$), 7.14 (2 H, d, J = 7.8 Hz, H-3′,5′) 7.26 (1 H, t, J = 7.8 Hz, H-4′), 7.97 (1 H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) at δ: 14.24 (2 $\underline{CH_3}CH_2$), 24.60 (2 $\underline{CH_2}CH_3$), 42.59 $\underline{CH_2}Cl$), 126.31 (C-3′,5′), 128.28 (C-4′), 131.44 (C-1′), 141.21 (C-2′,6′), 165.01 (CO-NH). EI^+-MS: $[M]^+$ m/z: 225 (89.3%), calculated for $C_{12}H_{16}NOCl$. Other fragments at m/z = 190 (11.0%), 176 (100%), 148 (77.9%), 147 (76.61%), 146 (41.5%), 132 (70.9%), 120 (60.9%), 117 (70.3%), 105 (28.6%), 103 (22.9%), 91 (57.5%), 77 (47.2%), 65 (19%), 35 (0.7%). HR EI^+-MS: calculated: 225.0920, found: 225.2200.

3.7. Compound 3

White powder (70 mg), $R_f = 0.43$ (system II), m.p. 85–87 °C, IR, υ_{max} (KBr), cm^{-1}: 3420, 2960, 2850 and 1470. ^1H NMR (400 MHz, $CDCl_3$) at δ: 0.86 (3 H, t, J = 6.8 Hz, CH_3), 1.25 (56 H, s, 28 CH_2), 1.58 (2 H, m, $\underline{CH_2}CH_2OH$), 3.64 (2 H, t, J = 6.8 Hz, $\underline{CH_2}OH$). ^{13}C NMR (100 MHz, $CDCl_3$) at δ: 14.11 (CH_3), 22.69 (CH_2), 25.73 (CH_2), 29.36 (CH_2), 29.43 (CH_2), 29.61 (CH_2), 31.92 (CH_2), 32.80 ($\underline{CH_2}CH_2OH$) and 63.10 (CH_2OH). EI^+-MS: m/z 438 (0.2%) calculated for $C_{30}H_{62}O$, other fragments at 420 (1.6%), 392 (7.8%), 364 (7.7%), 336 (2.0%), 308 (1.20%).

3.8. Compound 4

White crystalline needles (50 mg), $R_f = 0.58$ (system III). m.p. 203–205 °C, UV λ_{max} (EtOH): 230, 254, 260, 298, 364 nm. ^1H NMR ($CDCl_3$), 400 MHz) at δ: 3.95 (3 H, s, OCH_3), 6.17 (1 H, s, OH), 6.27 (1 H, d, J = 9.50 Hz, H-3), 6.85 (1 H, s, H-8), 6.92 (1 H, s, H-5), 7.60 (1 H, d, J = 9.50, H-4). ^{13}C NMR (100 MHz, $CDCl_3$) at δ: 56.4 (OCH_3), 103.18 (C-8), 107.47 (C-5), 111.48 (C-5a), 113.41 (C-3), 143.29 (C-4), 143.99 (C-7), 149.68 (C-8a), 150.24 (C-6), 161.43 (C-2).

3.9. Compound 5

White amorphous powder (200 mg), $R_f = 0.47$ (system III). m.p. 232–234 °C, IR υ_{max} (KBr), cm^{-1}, 3400, 2940, 1450, 1370, 1610, 1070 and 1020. ^1H NMR (C_5D_5N), 400 MHz) at δ: 0.65 (3 H, s, 18-CH_3), 0.85 (3 H, d, J = 6.00 Hz, 27-CH_3), 0.87 (3 H, t, J = 6.00 Hz, 29-CH_3), 0.89 (3 H, s, 19-CH_3), 0.90 (3 H, d, J = 6.00 Hz, 26-CH_3), 0.98 (3 H, d, J = 6.00 Hz, 21-CH_3), 1.00–2.75 (CH_2 and CH protons, m), 3.96 (1 H, m, H-3), 4.07–4.42 (sugar protons), 5.07 (1 H, d, J = 7.6 Hz, H-1′), 5.20 (2 H, d, J = 8.5 Hz, H-22, 23), 5.34 (1 H, d, J = 7.5 Hz, H-6). ^{13}C NMR (C_5D_5N), 100 MHz) at δ: 11.98 (C-18), 12.16 (C-29), 19.02 (C-19), 19.21 (C-26), 19.43 (C-27), 19.99 (C-21), 21.29 (C-11), 23.39 (C-15), 24.52 (C-16), 28.55 (C-28), 29.46 (C-7), 30.26 (C-2), 32.06 (C-8), 32.18 (C-25), 34.21 (C-10), 36.40 (C-1), 36.93 (C-4), 37.49 (C-20), 39.35 (C-13), 39.95 (C-12), 50.34 (C-9), 56.24 (C-14), 56.83 (C-17), 71.69 (C-3), 121.93 (C-23), 129.20 (C-22), 139.00 (C-5), 140.90 (C-6), 102.59 (C-1′), 75.36 (C-2′), 78.63 (C-3′), 78.09 (C-4′), 7 8.51 (C-5′), 62.84 (C-6′).

Acknowledgement: The authors thank Prof. Dr. A. El-Shorbagi (Department of Organic Pharmaceutical Chemistry, Faculty of Pharmacy, Assiut University, Egypt) for helpful discussions.

References

1 Khafagy, S. M.; Metwally, A. M.: J. Pharm. Sci. UAR **9**, 83 (1968)
2 Rojo, H. P.; Quiroga E. N.; Vattuone, M. A.; Sampietro, A. R.: Phytochemistry **49**, 965 (1998)
3 Khafagy, S. M.; Metwally, A. M.: J. Pharm. Sci. UAR **9**, 117 (1968)
4 Morel, A. F; Machado, E. C.; Navarro, C. E.; Giacomelli, S. R.; Monachi, F. D.: Planta Med. **64**, 284 (1998)
5 Uegaki, R.; Fujimori, T.; Kubo, S.; Kato, K.: Phytochemistry **20**, 1567 (1981)
6 Maier, W; Scmidt, J.; Nimtz, M.; Wray, V.; Strac, D.: Phytochemistry **54**, 473 (2000)
7 Saitoh, F.; Noma, M.; Kawashima, N.: Phytochemistry **24**, 477 (1985)
8 Snook, M. E.; Chortyk, O. T.; Sisson, V. A.; Coserllo, C.E.: Phytochemistry **31**, 1639 (1992)
9 Breitmaier, E.; Voelter, W.: Carbon-13 NMR Spectroscopy. 3rd edn., VCH, Weinheim, Germany 1987
10 Solomons, T. W. G.; Organic Chemistry. 6th edn. p. 618, John Wiley & Sons, Inc., New York, Toronto, Singapore, 1996
11 Kalianos, A. G.; Rec. Adv. Tob. Sci. **2**, 61 (1976)
12 Shinozaki, Y.; Matsuzaki, T.; Suhara, S.; Tobita, T.; Shigematsu, H.; Koiwai, A.: Agric. Biol. Chem. **55**, 751 (199471)
13 Harlan, W. R.; Moseley, J. M.; Tobaco, in Kirk-Othmer Encyclopedia of Chemical Tecnology, vol. 14, pp. 242–261, John Wiley & Sons, New York 1955
14 Stahl, E.: Thin layer chromatography, Student Edition, p. 892, Springer-Verlag, Berlin, Heidelberg, New York 1969
15 Hannun, Y. A.; Obeid, L. M.; Dbaibo, G. S.: Handbook of Lipid Research vol. 8, pp. 177–204 (1996)
16 Dembitsky, V. M.; Shkrob, I.; Roze ntsvent, O.: Phytochemistry **54**, 965 (2000)
17 Friebolin, H.: One and two dimensional NMR spectroscopy, p. 133, VCH, Weinheim 1991
18 Ballantyne, M. M.; McCabe, P. H.; Murray, R. D. H.: Tetrahedron **27**, 871 (1971)
19 El-Sayyad, S. M.; Abdel-Baky, A. M.; Backheet, E. Y.; Glombitza K.-W.: Bull. Pharm. Sci., Assiut Univ. **12**, 195 (1989)

Received August 13, 2001
Accepted September 15, 2001

Dr. Enaam Y. Backheet, Ph. D.
Department of Pharmacognosy
Faculty of Pharmacy
Assiut University
Assiut
71526 Egypt
enaam@acc.aun.eun.eg