Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 47 (2006) 7047–7050

The first example of natural cyclic carbonate in terpenoids

Sergio Rosselli, Antonella Maggio, Gabriella Bellone and Maurizio Bruno*

Dipartimento di Chimica Organica 'E. Paternò', Università di Palermo, Viale delle Scienze, Parco d'Orleans II, 90128 Palermo, Italy

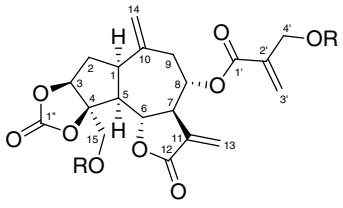
Received 23 June 2006; revised 18 July 2006; accepted 20 July 2006
Available online 10 August 2006

Abstract—The first natural occurring cyclic carbonate terpenoid, the guaianolide hololeucin (**1**), was isolated from the aerial part of *Centaurea hololeuca*. Its structure was elucidated on the basis of extensive proton, ^{13}C and two-dimensional NMR experiments, as well as by transformation in its diacetyl derivative.

As part of our ongoing chemical investigation of *Centaurea* species of the Mediterranean area,[1–4] we report on the isolation, from the aerial parts of *Centaurea hololeuca* Boiss., of a new guaiane sesquiterpene (**1**), carrying a cyclic carbonate. To our knowledge, this is the first example in the literature of cyclic carbonate terpenoid. The genus *Centaurea* L. (Asteraceae, tribe Cardueae, subtribe Centaureinae) comprises ca. 600 species distributed in Asia, Europe, North Africa and America[5,6] and previous chemical studies indicate that patterns of secondary metabolites present in plants of this taxon include triterpenes, steroids, hydrocarbons, polyacetylenes, flavonoids, anthocyanins, lignans, alkaloids and sesquiterpenoids.[7] Among the latter, guaianes, germacranes and elemanes are the most common and seem to have systematical importance within the genus *Centaurea*. Guaianes and guaianolides have attracted interest due to their peculiar biological activities such as cytotoxic,[8–12] antibacterial,[13] tripanocidal,[14] *anti*-ulcerogenic[15] and allelopathic.[16]

Recently, we reported on the phytochemical investigation of the aerial parts of *Centaurea hololeuca* Boiss. and the isolation of seven known guaianolides: repin, cynaropicrin, janerin, cebellin G, babylin B, cebellin J and 15-deschloro-15-hydroxychlorojanerin.[17]

The most polar fraction of the main column chromatography, eluted with EtOAc–MeOH 19:1, was purified by repeated column chromatography to give a subfraction that, after several washings with $CHCl_3$, allowed us to isolate a pure, white, amorphous solid (compound **1**, 15 mg), insoluble in several organic solvents (petrol, CH_2Cl_2, EtOAc, $CHCl_3$) but soluble in acetone.



1 R = H
2 R = Ac

Compound **1**, molecular formula $C_{20}H_{22}O_9$, showed in its IR spectrum absorptions for hydroxyl 3528 and 3300 cm^{-1}, γ-lactone (1767), ester (1710, 1209) and olefinic (1665, 1635) groups. Its complete structure and stereochemistry were elucidated by the use of ^1H NMR, ^{13}C NMR and several two-dimensional techniques (Table 1). By extensive ^1H NMR decoupling experiments, it was possible to achieve several information. In fact irradiation at the frequencies of the typical doublets of the exocyclic methylene group of the γ-lactone ring (δ 6.14 and δ 5.82) identified H-7 as a dddd at δ 3.55. Further irradiation at the latter frequency collapsed the H-6 dd at δ 4.51 to a doublet and affected the overlapped signal at δ 5.21, consequently assigned to H-8. Irradiation at the frequency of H-6 permitted the identification of H-5, a dd at δ 2.73 which coupled with the ddd at δ 3.33 that, therefore, was identified as H-1. Further selective decouplings allowed to identify H-2α and H-2β ddds at δ 2.48 and δ 2.31, respectively, and H-3, overlapped signal at δ 5.19. On the other hand, irradiation at the frequency of H-8 collapsed the mutually coupled dd at δ 2.98 and ddd at δ 2.33 (H-9β and

* Corresponding author. Tel.: +39091596905; fax: +39091596825; e-mail: bruno@dicpm.unipa.it

0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2006.07.099

Table 1. Spectral data for **1** and **2**

C/H no.	**1**[a]				**2**[b]			
	δ_H mult. (*J* in Hz)[c,e]	NOESY	δ_C[c,f]	HMBC (H)	δ_H mult. (*J* in Hz)[c]	δ_H mult. (*J* in Hz)[d]	δ_C[c,f]	δ_C[d,f]
1α	3.33 ddd (9.6, 8.4, 4.0)	3, 5, 7, 2α	47.12 d	3, 14a, 14b	3.39 ddd (9.6, 8.7, 4.5)	3.07 m	46.91 d	45.68 d
2α	2.48 ddd (15.6, 8.4, 7.2)	2β, 1	37.94 t		2.56 ddd (15.6, 8.7, 7.2)	2.35 m	37.98 t	36.62 t
2β	2.31 ddd (15.6, 4.0, 2.4)	2α			2.34 ddd (15.6, 4.5, 2.7)	2.35 m		
3α	5.19[g]	1, 2α	85.13 d		5.22 dd (7.2, 2.7)	4.95 dd (7.2, 2.7)	85.31 d	83.86 d
4			95.65 s				93.32 s	91.81 s
5α	2.73 dd (10.4, 9.6)	7, 1	52.68 d	15b, 7	2.89 dd (10.4, 9.6)	2.47 dd (10.4, 9.6)	53.19 d	52.31 d
6β	4.51 dd (10.4, 8.8)	8	77.32 d	5	4.57 dd (10.4, 8.4)	4.39 dd (10.4, 8.4)	76.96 d	75.39 d
7α	3.55 dddd (10.0, 8.8, 3.2, 2.8)	1, 5	47.53 d	13a, 13b, 9β, 5, 9α	3.58 dddd (9.6, 8.4, 3.3, 2.7)	3.26 dddd (9.6, 8.4, 3.3, 2.7)	47.45 d	46.57 d
8β	5.21[g]	6	75.45 d	6, 9β, 9α	5.27 ddd (9.9, 9.6, 5.4)	5.07 ddd (9.9, 9.6, 5.4)	75.82 d	74.10 d
9α	2.33 ddd (13.2, 9.6, 1.2)	9β	44.50 t		2.38 dd (13.5, 9.9)	2.12 dd (13.5, 9.9)	44.14 t	44.43 t
9β	2.98 dd (13.2, 5.6)	9α, 14b			2.98 dd (13.5, 5.4)	2.93 dd (13.5, 5.4)		
10			144.70 s	9β, 5, 2α, 9α, 2β			144.29 s	140.65 s
11			138.48 s	13a			138.42 s	135.01 s
12			169.89 s	13a			169.70 s	168.12 s
13a	6.14 d (3.2)		124.43 t		6.16 d (3.3)	6.27 d (3.3)	124.36 t	124.43 t
13b	5.82 d (2.8)				5.79 d (2.7)	5.76 d (2.7)		
14a	5.18 d (1.2)		118.04 t	9β, 9α	5.18 br s	5.18 br s	118.36 t	118.70 t
14b	5.12 br s	9β			5.15 br s	5.17 br s		
15a	4.09 dd (12.0, 5.2)	15b	65.38 t	5	4.58 d (12.6)	4.54 d (12.6)	66.90 t	65.07 t
15b	3.62 dd (12.0, 5.2)	15a			4.25 d (12.6)	4.18 d (12.6)		
1′			166.09 s	3′a			165.41 s	163.73 s
2′			142.57 s	3′a			137.44 s	135.09 s
3′a	6.28 d (1.6)		125.62 t		6.41 br s	6.37 br s	129.48 t	129.19 t
3′b	6.00 d (1.6)				6.03 br s	5.92 br s		
4′ (2H)	4.33 br d (5.6)		61.92 t	3′a, 3′b	4.82 br s	4.76 br s	63.47 t	62.24 t
1″			155.36 s	3			154.84 s	153.05 s
15-OH	4.71 t (5.2)				—	—	—	—
4′-OH	4.22 t (5.6)				—	—	—	—
OAc					2.06 s	2.06 s	21.35 q	20.81 q
							171.13 s	170.24 s
OAc					2.04 s	2.04 s	21.14 q	20.57 q
							171.13 s	170.24 s

Data for compound **1**: $[\alpha]_D^{25}$ +86.1 (*c* 0.20, MeOH); IR (film) 3528, 3300, 2930, 2852, 1782, 1767, 1710, 1665, 1635, 1460, 1377, 1300, 1209, 1163, 1143, 1060 cm^{-1}; ESIMS (pos. mode) 455 $[M + K]^+$ (45), 429 $[M+Na]^+$ (100); ESIMS (neg. mode) 441 $[M+Cl]^-$ (100); Anal. Calcd for $C_{20}H_{22}O_9$: C, 59.11; H, 5.46. Found C, 59.09; H 5.48.

Data for compound **2**: $[\alpha]_D^{25}$ +73.4 (*c* 0.44, $CHCl_3$); IR (film) 2937, 2854, 1801, 1782, 1771, 1747, 1643, 1450, 1367, 1301, 1267, 1232, 1142, 1076 cm^{-1}; ESIMS (pos. mode) 529 $[M+K]^+$ (100), 513 $[M+Na]^+$ (57), 491 $[M+H]^+$ (10); ESIMS (neg. mode) 525 $[M+Cl]^-$ (100); Anal. Calcd for $C_{24}H_{26}O_{11}$: C, 58.77; H, 5.34. Found C, 58.80; H 5.32.

[a] 400 MHz for ^1H and 100 MHz for ^{13}C NMR.

[b] 300 MHz for ^1H and 75 MHz for ^{13}C NMR.

[c] Acetone-d_6.

[d] $CDCl_3$.

[e] After addition of D_2O, the signals at δ 4.71 and 4.22 disappear and the signals at δ 4.33, 4.09 and 3.62 collapse in br s, d and d, respectively.

[f] Multiplicity has been determined by DEPT experiments and assignments have been made by HSQC.

[g] Overlapped signal.

H-9α) to a broad doublet and a dd, respectively. Irradiation at these two latter frequencies sharpened the broad singlet at δ 5.12 and collapsed the doublet at δ 5.18 into a singlet (H-14a and H-14b). The presence of a hydroxymethylene was confirmed by the two mutually coupled double doublets at δ 4.09 and δ 3.62 (H-15a and H-15b) that collapsed in two doublets after addition of D_2O. The coupling constants involving H-1, H-2α, H-2β, H-3, H-5, H-6, H-7, H-8, H-9β and H-9α as well as the comparison with literature data[17] established the relative stereochemistry of the guaiane moiety.

The nature of the side chain was indicated by the signals, in the ^1H and ^{13}C NMR, at δ_C 166.09 s (C-1′), δ_C 142.57 s (C-2′), δ_C 125.62 t (C-3′), δ_H 6.28 d and δ_H 6.00 d (H-3′a and H-3′b), and δ_C 61.92 t (C-4′) and δ_H 4.33 br d (2H-4′), the latter collapsing into a broad singlet after addition of D_2O. Registration of ^{13}C NMR, DEPT and HSQC spectra allowed us to identify all the carbons of the guaiane skeleton but showed the presence of an additional, unexpected signal at δ_C 155.36 s, typical for a cyclic carbonate,[18–20] that must be linked to C-3 and C-4, as clearly shown by downfield chemical shifts of C-3 and C-4 at δ_C 85.13 d and δ_C 95.65 s, respectively. This fact was clearly confirmed by the HMBC spectrum, that showed, among others, a clear correlation peak between the carbonyl at δ_C 155.36 and the proton H-3 at δ_H 5.19. The stereochemistry of the carbonate cycle was confirmed to be β by a NOESY experiment. In fact proton H-1 at δ 3.33 showed clear correlations with H-3, H-5, H-7 and H-2a. Consequently, to compound **1** was assigned the structure depicted in the formula and the trivial name of hololeucin. A related natural guaianolide without carbonate functionality but with the same C3–C4 stereochemistry has been previously isolated from *Centauries pseudosinaica*.[21] Now the unusual polarity of this compound, in despite of the presence of only two hydroxyl groups, can be ascribed to the highly polar carbonate moiety.[22]

The presence in compound **1** of only two hydroxyl groups on C-15 and C-4′ was early confirmed by the treatment of hololeucin with a mixture of (1:1) Ac_2O-pyridine. The solution was allowed to stand overnight at rt and, after the usual work-up, compound **2** was obtained in 95% yield. The IR spectrum of compound **2** (molecular formula $C_{24}H_{26}O_{11}$) was devoid of absorptions for hydroxyl groups and its ^1H NMR and ^{13}C NMR spectra (acetone-d_6) (Table 1) showed signals for two acetyl groups at δ_H 2.06 s, 2.04 s and δ_C 171.13 s (×2), 21.35 q and 21.14 q, linked to C-15 and C-4′, as clearly indicated by the downfield shifts of H-15a and H-15b at δ_H 4.58 d, 4.25 d, respectively, and of the two protons on C-4′ at δ_H 4.82 br s.

The occurrence of a cyclic carbonate moiety is quite rare in natural products. Examples concerning isolation of polyketides carrying cyclic carbonate from fungi were recently reported.[18,19] Another paper reported the isolation of a lignane with a cyclic carbonate group from an amazonic plant of *Strychnos* genus.[20] To our knowledge, no product was discovered in terpenoid series bearing a cyclic carbonate. Concerning the biological activity of this product, we reported that guaianolides with similar structure showed good cytotoxic activity.[12] However, preliminary cytotoxic tests against tumor cell replication (A549, 1A9, MCF-7 and PC-3) of compounds **1** and **2**, did not give good activity. This finding is in agreement with the poor cytotoxic activity reported for guaianolides having a 3β,15-dihydroxy moiety.[12]

Acknowledgements

The authors thanks Professor K.-H. Lee (Natural Products Laboratory—University of North Carolina at Chapel Hill) for bioassay. Financial support by Italian MIUR PRIN is gratefully acknowledged.

References and notes

1. Bruno, M.; Vassallo, N.; Fazio, C.; Gedris, T. E.; Herz, W. *Biochem. Syst. Ecol.* **1998**, *26*, 801–803.
2. Bruno, M.; Maggio, A.; Paternostro, M. P.; Rosselli, S.; Arnold, N. A.; Herz, W. *Biochem. Syst. Ecol.* **2001**, *29*, 433–435.
3. Bruno, M.; Maggio, A.; Rosselli, S.; Gedris, T. E.; Herz, W. *Biochem. Syst. Ecol.* **2002**, *30*, 379–381.
4. Bruno, M.; Rosselli, S.; Maggio, A.; Raccuglia, R. A.; Arnold, N. A. *Biochem. Syst. Ecol.* **2005**, *33*, 817–825.
5. Hickey, M.; King, C. J. *100 Families of Flowering Plants*; Cambridge University Press, 1981.
6. Heywood, V. H. *Flowering Plants of the World*; Oxford University Press, 1979.
7. Al-Easa, H. S.; Rizk, A. M. *Qatar Univ. Sci. J.* **1992**, *12*, 27–57.
8. Cho, J. Y.; Kim, A. R.; Jung, J. H.; Chun, T.; Rhee, M. H.; Yoo, E. S. E. *J. Pharmacol.* **2004**, *492*, 85–94.
9. Li, X.; Qian, P.; Liu, Z.; Zhao, Y.; Xu, G.; Tao, D.; Zhao, Q.; Sun, H. *Heterocycles* **2005**, *65*, 287–291.
10. Ha, T. J.; Jang, D. S.; Lee, J. R.; Lee, K. D.; Lee, J.; Hwang, S. W.; Jung, H. J.; Nam, S. H.; Park, K. H.; Yang, M. S. *Arch. Pharmacol. Res.* **2003**, *26*, 925–928.
11. Muhammad, I.; Takamatsu, S.; Mossa, J. S.; El-Feraly, F. S.; Walker, L. A.; Clark, A. M. *Phytother. Res.* **2003**, *17*, 168–173.
12. Bruno, M.; Rosselli, S.; Maggio, A.; Raccuglia, R. A.; Bastow, K. F.; Lee, K.-H. *J. Nat. Prod.* **2005**, *68*, 1042–1046.
13. Modonova, L. D.; Semenov, A. A.; Zhapova, T.; Ivanova, N. D.; Dzhaparova, A. K.; Fedoseev, A. P.; Kirdei, E. G.; Malkova, T. I. *Khimiko-Farmatsevticheskii Zhurnal* **1986**, *20*, 1472–1475.
14. Schinor, E. C.; Salvador, M. J.; Ito, I. Y.; De Albuquerque, S.; Dias, D. A. *Phytomedicine* **2004**, *11*, 224–229.
15. Yesilada, E.; Guerbuez, I.; Bedir, E.; Tatli, I.; Khan, I. A. *J. Ethnopharmacol.* **2004**, *95*, 213–219.
16. Stevens, K. L.; Merrill, G. B. *ACS Symp. Ser. (Chem. Allelopathy)* **1985**, *268*, 83–98.
17. Rosselli, S.; Maggio, A. M.; Raccuglia, R. A.; Simmonds, M. S. J.; Arnold, N. A.; Bruno, M. *Nat. Prod. Commun.* **2006**, *1*, 281–285.
18. Liu, Z.; Jensen, P. R.; Fenical, W. *Phytochemistry* **2003**, *64*, 571–574.
19. Rohan, A.; Davis, R. A.; Andjic, V.; Kotiw, M.; Shivas, R. G. *Phytochemistry* **2005**, *66*, 2771–2775.

20. Pinheiro, M. L. B.; Imbiriba da Rocha, A. F.; Fernandes, M. A. do. N.; Queiroz Monte, F. J.; Figueroa Villar, J. D.; Rangel Cruz, E. *Quim. Nova* **2004**, *27*, 188–192.
21. Amer, M. E.; Abdel-Kader, M. S.; Hassan, M. A.; Mossa, J. S.; El-Masry, S. *Alexandria J. Pharm. Sci.* **2001**, *15*, 65–67.
22. Webster, D. C. *Prog. Org. Coat.* **2003**, *47*, 77–86.