Tr. J. of Chemistry
20 (1996) , 329 – 334.
© TÜBİTAK

The Sterols of *Cyclamen Coum*

Nurettin YAYLI, Cemalettin BALTACI
Karadeniz Technical University, Faculty of Science,
Department of Chemistry 61080 Trabzon-TURKEY

Received 8.5.1996

The structures of one major and three minor constituents of sterol fraction of the *Cyclamen coum* have been established as stigmasterol [(22E)- (24S)- 24-ethylcholesta-$\Delta^{5,22}$-dien-β-ol] (**1**), Poriferasterol [22E)-(24R)-24-ethylcholesta-$\Delta^{5,22}$-dien-3β- ol] (**2**), stigma-or poriferasta-$\Delta^{3,5,22}$ triene [(22E)-24-ethylcholesta-$\Delta^{3,5,22}$-trien] (**3**) stigma-or poriferosta- $\Delta^{3,5,7,22}$-tetraene [(22E)-24-ethylcholesta-$\Delta^{3,5,7,22}$-tetraene] (**4**) by spectroscopic means (NMR, EI-MS and GC-MS).

Introduction

Cyclamen coum is widely distributed in the north of Turkey. Prior to the initiation of this study, it was found that there were no chemical studies of *Cyclamen coum* in the literature. This paper describes the isolation and structure elucidation of sterols compounds from *Cyclamen coum.*

The occurrence of sterols in plants and marine organisms is well established[1–17]. Several such compounds are reported in the literature[1–17]. Analysis of one of the apolar fraction by chromatographic methods from *Cyclamen coum* gave compound **1**. Its structure was established principally by nmr methods and EI-MS.

Experimental Section

General

Nmr spectra were recorded on a Bruker AC 200L NMR at 200 MHz in $CDCl_3$ using TMS as internal standard. IR spectra were taken on a Perkin Elmer 1600 spectrophotometer. Mass spectra were recorded on a Zabspec EI-MS instrument. melting point was determined on a Gallenkamp apparatus and uncorrected. GC-MS was recorded on a HP 5890 Gas Chromatography with He carrier gas and Zabspec mass spectrometer with EI source, trisector double focusing equipped with a megabore capillary column at Marmara Research Center, Instrumental Analysis Laboratories, Gebze. The optical rotation was measured on a full-circle model of Polyscience Polarimeters using a 20x1 cm cell. Flash column chromatography was performed on a silica gel 60 (230-400 mesh) and preparative tlc was performed with precoated silica gel F_{254} (20x20 cm, 0.2 mm) plates. A voucher specimen was deposited in deepfreeze at the Department of Chemistry, Karadeniz Technical University.

Isolation

Specimens of *Cyclamen coum* were collected in the Giresun Yağlıdere region, in the north of Turkey in March, 1995. The chopped wet plants (~1500 g) were extracted with cold CH_3OH (1.5 lt, 3 times, 24 hours each). The total aqueous CH_3OH extract was filtered, and the filtrate was concentrated on a rotary evaporator at 30° C. The aqueous extract thus obtained (0.4 liter) was extracted with $CHCl_3$ (150 ml, 3 times). After collecting $CHCl_3$ extract (450 ml), it was evaported *in vacuo* at 30-35 °C. The crude mixture (0.9 g) was chromatographed on a Kieselgel 60 (40 g, 230-400 mesh) flash column chromatograph Elution with n-hexane, then discontinuous gradient elution with n-hexane-$CHCl_3$ (3:1-1:4) and $CHCl_3$ and the discontinuous gradient elution with $CHCl_3 - CH_3OH$ (9:1-2:3) and finally with $CHCl_3 - CH_3OH - H_2O$ (2:2.6:0.4) gave 43 fractions (*ca.* 15-20 ml each). Fractions 14-22 were combined after the analyses of tlc to obtain the fourth fraction. Evaporation of the fourth fraction was rechromatographed on the Kieselgel 60 column and gave an amorphous material (69.2 mg) showing R_f 0.9-0.7 on silica gel tlc using $CHCl_3 - CH_3OH$ (4:1). This material was further purified on a column of Kieselgel 60 (10 g, 230-400 mesh) eluting with n-hexane, and then discontinuous gradient of n-hexane-$CHCl_3$ (5:1-2:1) to give 24 fractions (ca. 3-5 ml each). Fractions 10-16 were combined and evaporated to give acrude mixture (22 mg). A portion of this mixture (15 mg) was further purified on preparative tlc plates (20x20 cm, 2 plates, 0.2 mm) $CHCl_3 - CH_3OH$ (3:0.2, $R_f = 0.5$) Using the solvent system to give compound **1**. This was crystallized in methanol solution to obtain a pure compound **1** (7.2 mg). GC-MS spectra of a portion of the crude mixture (7 mg), obtained from combining fractions 10-16, was taken and gave compounds **2,3** and **4**.

(24S)-Stigmast-5,22(E)-dien-3β-ol 1: M.p.=149- 151° C; $[\alpha]^{23}$ D-48 (c=0.0002, $CHCl_3$); EI-MS m/z (%): 412(22), 413(6), 397(20), 394(100), 351(13), 300(10), 271(16), 255(38) and further peaks at m/z 213, 159, 145, 133, 105, 95, 83 and 63 common to stigmasterol and related steroids; GC-MS m/z (%): 412(26), 413(8), 397(4), 394(9), 379(7), 351(13), 300(16), 271(25), 255(38) and further peaks at m/z 213, 199, 159, 133, 119, 105, 91, 83, 69, 55, 43, 29; IR ($CHCl_3$) (cm^{-1}): 3440-3410, 3020-3076, 1640-1652, 1360-1372, 950, 848.

(24R)-Poriferast-5,22 (E)-dien -3β-ol 2: GC-MS m/z (%): 412(12), 413(3), 397(5), 394(2), 379(2), 369(5), 351(4), 300(10), 271(82), 255(36) and further peaks at m/z 229, 213, 173, 159, 147, 133, 119, 107, 105, 83, 81, 69, 55, 43, 29, 28.

Stigmast-or Poriferast -3,5,22(E)-trien 3: GC-MS m/z (%): 395(13), 396(1), 379(4), 351(6), 282(6), 267(6), 255(38) and further peaks at m/z 228, 213, 199, 159, 145, 133, 105, 93, 83, 81, 69, 55, 43, 29, 28.

Stigmast-or Poriferast -3,5,7,22 (E)-tetraene 4: GC-MS m/z (%): 392(24), 392(24), 293(5), 253(10), 211(4) and further peaks at m/ z 207, 189, 157, 143, 135, 119, 105, 91, 83, 69, 55, 29, 28.

Results and Discussion

We determined compound **1** to be stigmasterol based on the following evidence. The EI-MS spectrum of stigmasterol displays molecular ions at m/z 412 $[M]^+$, $413[M+1]^+$, $414[M+2]^+$, $397[M\text{-}CH_3]^+$ and $394[M\text{-}H_2O]^+$ in support of formulation **1**. The APT nmr ($CDCl_3$) shows the presence of 28 carbons. However, the height of peak at δ 31.9 ppm is almost twice the others. Therefore, it is assigned to be two peaks with total of 29 carbon atoms whose chemical shifts are entirely consistent with compound [1–4,9,12–24] **1** (see Table 1). The APT analysis of compound **1** showed six methyls, nine methylenes and eight methines signal. In the down field region of the APT spectrum there are four peaks, at δ 140.7 (C) 139.3(CH) 129.5(CH) and 121.7(CH) ppm assignable to two olefinic bonds which are located at Δ^5 and Δ^{22} positions of compound[1,12–15,20–22] **1**. This idea was also supported the presence of a doublet (1H) at δ 5-4 ppm and AB quartet (2H) at 5.1 ppm in the ^{1}H-nmr. From the 2D-COSY spectrum, it is possible to elucidate the connectivity of proton signals of compound **1** (see Table 1).

Table 1. NMR data for stigmasterol **1**, $(CDCl_3)^a$

Carbon No.	^{13}C(δ, ppm)^b	APT	^{1}H(δ, ppm) ^{c}, J(Hz)
1	37.3	CH_2	
2	28.9	CH_2	2.3
3	71.8	CH_2	3.52 (m,1H)
4	42.3	CH_2	2.3
5	140.7	C	
6	121.7	CH	5.38 (1H,bd), J=6 Hz
7	31.9	CH_2	2.0
8	31.9	CH	
9	50.2	CH	
10......	39.7	C	
11......	24.4	CH_2	
12......	29.7	CH_2	
13......	39.7	C	
14......	56.7	CH	
15......	25.5	CH_2	
16......	29.7	CH_2	
17......	56.0	CH	
18......	12.1	CH_3	0.7 (3H,s)
19......	12.2	CH_3	1.1 (3H,s)
20......	40.5	CH	
21......	21.2	CH_3	1.1 (3H,d)
22......	139.3	CH	5.1 (AB, q, 1H)
23......	129.3	CH	5.1 (AB, q, 1H)
24......	51.3	CH	2.1
25......	31.9	CH	2.2
26......	19.4	CH_3^d	0.84 (3H,d), J=6.5 Hz
27......	19.0	CH_3^d	0.82 (3H,d), J=6.5 Hz
28......	29.7	CH_2	
29......	12.0	CH_3	0.84 (3H, t), J=7.2 Hz

[a] Chemical shifts (ppm) are relative to internal TMS.

[b] Some of the ^{13}C peaks may interchange.

[c] Assignments assisted by COSY data.

[d] Refrence 22, 23, 25

Furthermore, in the ^{1}H-nmr spectrum presence of a multiple (1H) peak at δ 3.5 ppm is characteristic H_3 of the compound.

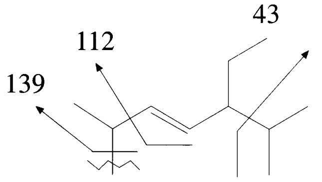
The optical rotation of compound **1** was measured in $CHCl_3$, and found to be $[\alpha]^{23}$-48, which was in agreement with the literature value of stigmasterol[27].

Therefore, comparison of all the spetral data with those published for related compounds[1–4,9,12–24] isolated from defferent sources showed that compound **1** was isolated from *Cyclamen coum* for the first time in pure from.

The chloroform soluble portions obtained from the methanol extracts of the fresh *Cyclamen coum* evaporated and a crude mixture was obtained. This was subjected to silica gel flash column chromatography to give one of the apolar fraction of crude sterols, from which compound **1** is purely isolated by further chromatographic methods. Tlc analysis of this crude fraction indicated that it contains stigmasterol and related apolar comounds. In order to identify related compounds, GC-MS spectrum of the crude fraction was taken. Three other minor compounds in the ratio of 33:4: 3:1 (**1-4**) were found in the fraction.

In the GC-MS spectra retenetion time of the peak was 50.984 and displayed molecular ions at m/z 412 $[M]^+$, $413[M+1]^+$, $414[M+2]^+$, $397[M\text{-}CH_3]^+$, 394 $[M\text{-}H_2O]^+$, 351 $[M\text{-}H_2O - 43]^+$, 271 $[M\text{- }141]^+$ and 255 $[M\text{-}H_2O\text{-}139]^+$ (see table 2), in support of formulation $C_{29}H_{48}O$. In the GC-MS spectra, relative ratio of the peak was 100 % and it was the major compound of crude fraction. Since, compound **1** was isolated from one portion of the crude mixture, the major peak in the GC-MS spectra was also assigned to be stimasterol [8,10,11] **1**.

Table 2. Fragmentation Patterns from GC-MS Spectra of *Cyclamen coum.*

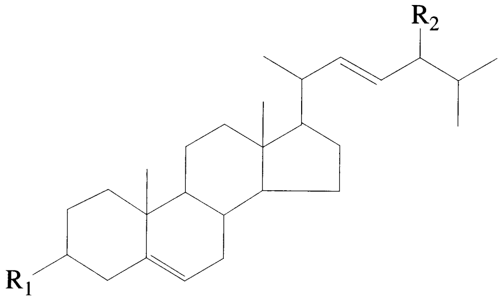


Steroid	m/z	Relative Abundance
Stigmasterol	$412[M]^+$	26
	$413[M+1]^+$	8
	$414[M+2]^+$	3
	$397[M\text{-}CH_3]^+$	4
	$394[M\text{-}CH_2O]^+$	9
	$379[M\text{-}H_2O - CH_3]^+$	7
	$369[M\text{-}C_{25} - C_{27}]^+$	5
	$351[M\text{-}H_2O - 43]^+$	13
	$300[M\text{-}112]^+$	16
	$271[M\text{-}141]^+$	25
	$255[M\text{-}H_2O - 139]^+$	38
Poriferasterol	$412[M]^+$	12
	$413[M+1]^+$	3
	$397[M\text{-}CH_3]^+$	5
	$394[M\text{-}H_2O]^+$	2
	$379[M\text{-}H_2O - CH_3]^+$	2
	$369[M\text{-}C_{25}\text{-}C_{27}]^+$	5
	$351[M\text{-}H_2O - 43]^+$	4
	$300[M\text{-}112]^+$	10
	$271[M\text{-}141]^+$	82
	$255[M\text{-}H_2O - 139]^+$	36
Stigma-or poriferasta-3,5,22 triene	$394[M]^+$	38
	$395[M+1]^+$	13
	$396[M+2]^+$	1
	$379[M\text{-}CH_3]^+$	4
	$351[M\text{-}43]^+$	6
	$282[M\text{-}112]^+$	6
	$267[M\text{-}CH_3\text{-}112]^+$	3
	$255[M\text{-}139]^+$	38
Stigma-or poriferasta-3,5,7,22-tetraene	$392[M]^+$	24
	$393[M+1]^+$	5
	$253[M\text{-}139]^+$	10
	$211[M\text{-}181]^+$	4

In the GC-MS spectra retention time, the second peak was 51.571 and displayed molecular ions peak as the same as retention time 50.984 peak at m/z 412 $[M]^+$, 413 $[M+1]^+$, 397 $[M\text{-}CH_3]^+$, 394 $[M\text{-}H_2O]^+$, 271 $[M\text{-}141]^+$ and 255 $[M\text{-}H_2O-139]^+$, in support of formulation $C_{29}H_{48}O$, which is an isomer of compound **1**. In reviewing the literature findings[9–10,15,25–26], isomer form of compound **1** differs in C_{24}-ethyl stereochemistry. Stigmasterol has S configuration at C_{24}-ethyl position and the other isomer has R configuration[9–10,15,25]. Therefore, compound **2** is assigned to be poriferasterol, which was also isolated from *Cyclamen coum* for the first time.

The third peak of GC-MS spectra retention time was 48.087 and the relative ratio was 9 % and displayed molecular ions at m/z 394 $[M]^+$, 395 $[M+1]^+$, 396 $[M+2]^+$, 397 $[M\text{-}CH_3]^+$ 351 $[M\text{-}43]^+$, 267 $[M\text{-}CH_3-112]^+$ and 255 $[M\text{-}139]^+$ (see Table 1) in support of formulation $C_{29}H_{46}$, which differs 18 units (H_2O) from compound **1** and **2**. Thus, it is concluded that this compound has a third double band at C_{3-4} position by loss of H_2O. Therefore, compound 3 was stigma or poriferasta $\Delta^{3,5,22}$ triene which was isolated from *Cyclamen coum* for the first time and mentioned in the literature. In the literature however, it is reported that compound 3 is synthesized from stigmasterol by its oxidation[17].

The foruth peak of the GC-MS spectra of *Cyclamen coum*'s retention time was 47.794 and showed 3 % relative ratio and displayed molecular ion peak at m/z 392 $[M]^+$, 393 $[M+1]^+$ and 253 $[M\text{-}139]^+$ (see table 2), in support of formulation $C_{29}H_{44}$, which differes two units from compound **3**. That would suggest that compound **4** has a fourth double bond which was assigned to Δ^7 position due to conjugation[98,14,20–22]. Thus, compound **4** was assigned to be stigma-or poriferasta-$\Delta^{3,5,7,22}$ tetraene, which was mentioned in the pertinent literaure for the first time.



1 R_1 = OH, R_2 = ⋯ Et

2 R_1 = OH, R_2 = ◀ Et

3 R_1 = H, R_2 = –Et, Δ 3,5,22 triene

4 R_1 = H, R_2 = –Et, Δ 3,5,7,22 tetraen

Acknowledgment

This study was supported by a grant from the Karadeniz Technical University of Türkiye. Thanks to TUBITAK for recording the NMR spectra, the EI-MS and the GC-MS spectra, and to Dr. Mahir Küçük for collecting and identifying the organism.

References

1. S. Bano, U. V. Ahmad, S. Perveen, N. Bano, and M. Shameel, **Planta Medica, 18**, 117 (1986).

2. Y. Asakawa, M. Toyato and J. Harrison, A. Isotachin and B. Isotachin, **Phytochemistry 24,** 1505 (1985).
3. C. V. Shah, A. S. D'sa, and N. J. Sousa, **Steroids, 53,** 559 (1989).
4. F. Bohlmann, P. Singh and J. Jakupovic, **Phytochemistry, 21,** 2531 (1982).
5. Y. Ragasa, G. Padolina and Y. Otsuka, **Phytochemistry, 33,** 627 (1993).
6. A. Ulubelen, N. Gören and N. Tanker, **Journal of Natural Products, 48,** 1006 (1985).
7. H. M. Kazmi, A. Malik, S. Hameed, N. Akhtar and S. N. Ali, **Phytochemistry, 36,** 761 (1994).
8. Y. Nakajima, Y. Satoh, M. Katsumata, K. Tsujigma and J. Shoji, **Phytochemistry, 36,** 119 (1994).
9. F. Marie, C. Costet, L. Chapuis, R. Scalla and J. P. Delbecgue, **Plant Sciens, 91,** 23 (1993).
10. J. A. Ballantine and K. Williams, **Tetrahedron Letters, 18,** 1547 (1977).
11. B. Ngadjui, J. Ayofor, B. L. Sondemgam, J. D. Connolly and D. S. Rycroft, **Tetrahedron, 47,** 3555 (1991).
12. M. Henry and I. D. Chantalat, **Planta Medica,** 322 (1985).
13. P. A. Ramaiah, D. Lauie, R. D. Budhiraja, S. Sudhir and K. N. Garg, **Phytochemistry, 23,** 143 (1984).
14. A. S. R. Anjaneyulu, K. V. S. Raju, U. V. Mallavadhani and C. V. S. Prakash, **Indian Journal of Chemistry, 32B,** 457 (1993).
15. N. Jahan, W. Ahmed and A. Malik, **Journal of Natuarl Products, 58,** 1244 (1995).
16. L. J. Goad F. X. Garneau, J. L. Simard, J. W. Apsimon and M. Girard, **Comp. Biochem, physiol., 84B,** 189 (1986).
17. G. Blekas and D. Boskou, **Food Chemistry, 33,** 301 (1989).
18. L. G. Partridge, I. Midgley and C. Djerassi, **J. Am Chem Soc, 99,** 7686 (1977).
19. R. A. Laine and A. D. Elbein, **Biochemistry, 10,** 2547 (1971).
20. W. Voelter and E. Breitmaier, **"Carbon-13 NMR Spectroscopy"**, Third Edition, pp. 324-400, VCH Verlagsgesellschaft mbH, Weinheim, 1990.
21. P. Clerc and S. Simon, **"Spectral Data for Structure Determination of Organic Compounds"**, Second Edition, Color-Druek Dorfi GmbII, Berlin, 1989.
22. J. W. Blunt and J. B. Stothers, **"^{13}C NMR Spectra of Steroids", Organic Magnetic Resonance, 9,** 442 (1977).
23. A. E. Derome, **"Modern NMR Techniques for Chemistry, Research"**, Pergomon Press, 1988.
24. M. E. Rose and R. A. W. Johnstone, **"Mass Spectrometry For Chemists and Biochemists"** Cambridge University, 1982.
25. N. Rendell, N. L. A. Misso and J. Goad, **Lipids, 21,** 63 (1986).
26. N. L. Eatough, **Environ Inst., 15,** 19 (1989).
27. M. Windholz, **"The Merck Index"**, Merck and Co., Inc. Rahway, N. J., U.S.A, 1983.