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CONFIGURATION AT C-24 OF STEROLS FROM THE MARINE PHANEROGAMES *POSIDONIA OCEANICA* AND *CYMODOCEA NODOSA*

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Abstract—Sterols were extracted from two marine phanerogames, *Posidonia oceanica* and *Cymodocea nodosa* The two plants contain 24α-ethyl sterols, while the 24α-methyl sterols are accompanied by 24β-epimers The most abundant components are sitosterol, cholesterol and stigmasterol

INTRODUCTION

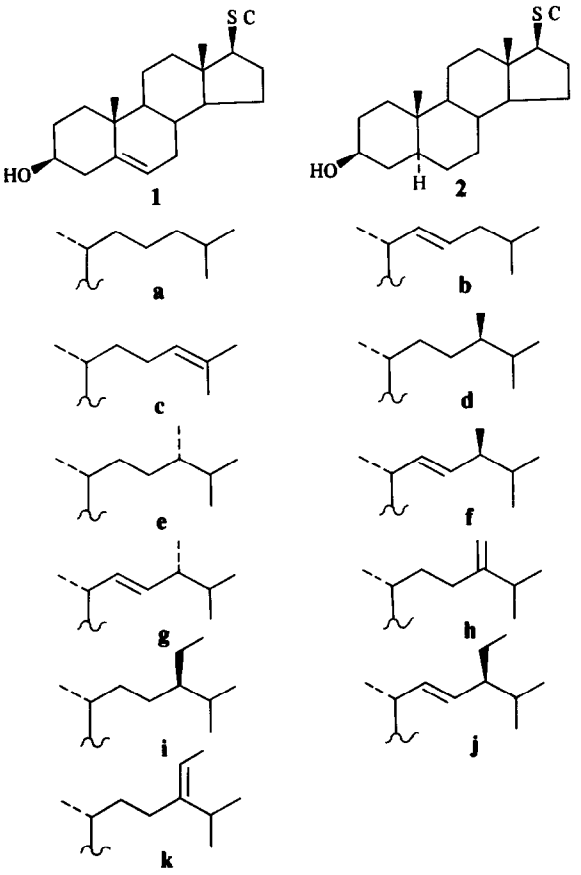
Terrestrial tracheophytes contain predominantly Δ^5 or Δ^7 C_{27}–C_{29} sterols In some cases stanols are present in low concentrations and some species contain relatively large quantities of $\Delta^{5,7}$-sterols The side chain may be saturated or, alternatively, may contain double bonds at C-22, C-24 (28), C-24, C-25 [1, 2] or C-23 [3, 4] Most algae and fungi contain sterols with the 24β-configuration, whereas higher plants produce predominantly 24α-ethyl sterols, 24α-methyl sterols are generally accompanied by 24β-epimers [5] Assignment of the configuration at C-24 has biosynthetic and chemotaxonomic significance [2]

The marine phanerogames are an ecologically important group of plants in the Mediterranean sea and may provide dietary sterols to other organisms Despite their importance in the marine food chain and the ease with which they can be collected, no mention exists about their sterol content Here we report the sterol composition and the stereochemistry of the C-24 alkylated sterols of two marine tracheophytes, *Posidonia oceanica* and *Cymodocea nodosa*, as a part of our work on marine sterols

RESULTS AND DISCUSSION

The unsaponifiable fraction of the crude extracts of the phanerogames was chromatographed on a silica gel column and the 4-demethylsterol fraction was acetylated and the steryl acetates were separated by argentation column chromatography and analysed by GC/MS The fractions were hydrolysed and the resulting free sterols were separated by reverse phase HPLC The configuration at C-24 of 24-alkyl sterols was determined by comparison with the ^{1}H NMR and ^{13}C NMR spectra of authentic compounds [6–8]

The fractions that contained cholesterol and its homologs yielded cholesterol, sitosterol (**1i**) and the two epimers at C-24, campesterol (**1d**) and 22,23-dihydrobrassicasterol (**1e**) This latter epimeric mixture had a ^{1}H NMR spectrum which was composed of signals from the two epimers The ^{1}H NMR spectrum of an authentic sample of campesterol supports the proposed configurations for **1d** and **1e** Estimation of peak heights of the C-27 and C-21 methyls established that **1d** and **1e** are in the approximate ratio of 2 3, for both phanerogames [9] The C-24 configuration of 24-ethylcholesterol was assigned by comparison of the ^{1}H NMR and ^{13}C NMR spectra with those of authentic sitosterol and clionasterol 24-Ethylcholestanol and 24-methylcholestanol possess the C-24 configurations observed for the corresponding Δ^5 sterols The former had a ^{1}H NMR spectrum identical to



that of catalytically hydrogenated sitosterol, while the latter exhibited a ^{1}H NMR spectrum similar to that of an epimeric mixture of 24-methylcholestanol, prepared by hydrogenation of 24-methylenecholestanol (**2h**)

The C_{28} $\Delta^{5,22}$-sterol fraction gave pure samples of epibrassicasterol (**1f**) and brassicasterol (**1g**) isolated by HPLC The two epimers can be distinguished by the differences in the chemical shift of the C-21 doublet in the ^{1}H NMR spectra which occurs at higher field in the spectrum of the faster eluted component **1f** [10] The presence of epibrassicasterol is reported in some marine organisms, where it co-occurs with its epimer at C-24 [11, 12], and recently was found in the seeds of *Brassica juncea* [13]

The sterol identified by GC and MS as 24-ethylcholesta-5,22-dien-3β-ol was purified by HPLC Its ^{1}H NMR spectrum was identical to that of an authentic sample of stigmasterol (**1j**)

P oceanica and *C nodosa* have rather similar sterol compositions (Table 1) The predominant sterols were identified as Δ^5 sterols and sitosterol is the major sterol Minor amounts of ring saturated sterols were also present The two plants contain 24α-ethyl sterols and 24α-ethyl stanols while the 24α-methyl sterols and 24α-methyl stanols are accompanied by the 24β-epimers

EXPERIMENTAL

Phanerogames *P oceanica* and *C nodosa* were collected in the Bay of Naples and supplied by the Zoological Station, Naples HPLC was carried out on a Waters instrument equipped with a differential refractometer, ^{1}H NMR (270, 500 MHz) and ^{13}C NMR (67 88 MHz), $CDCl_3$, TMS as internal standard, GC, SE-30 fused silica capillary column (30 m × 0 326 mm) at 250°, GC/MS, 70 eV

Extraction and separation The experimental procedure followed for *C nodosa* typifies the general procedure Freshly collected plants (800 g dry wt after extraction) were carefully washed and extracted with Me_2CO in a Soxhlet extractor for 24 hr The extract was saponified under reflux for 1 hr with 5% KOH in 80% EtOH The unsaponifiable lipids (3 g) were applied to a silica gel column which was eluted with CH_2Cl_2 The 4-demethylsterol fraction (0 55 g) was acetylated (Ac_2O–C_5H_5N, 16 hr at room temp) and the steryl acetates were purified by a silica gel column using as eluent petrol–Et_2O (96 4) Steryl acetates (0 5 g) were fractionated on an $AgNO_3$–silica gel (10 40) column which was eluted with increasing concns of C_6H_6 in petrol Fifteen fractions were collected and each analysed by GC/MS The fractions were saponified in 5% methanolic KOH under reflux for 20 min and the sterols were separated by reverse phase HPLC on a partisil ODS-2 column (250 × 4 6 mm) eluted with MeOH at 2 ml/min with 1 0 mg sterol mixture per injection

Table 1 Sterol composition (%) of phanerogames

Sterol	GC RR_t*	HPLC RR_t†	*C nodosa*	*P oceanica*
1b	0 91	0 74	0 7	1 9
2b	0 93	0 81	0 2	0 2
1a	1 00	1 00	16 4	17 0
2a	1 02	1 11	4 4	1 6
1c	1 08	0 78	0 2	trace
1f	1 11	0 84	2 4	1 6
1g	1 11	0 89	0 8	0 5
1h	1 26	0 78	0 6	1 7
2h	1 29	0 88	2 1	0 3
1d	1 29	1 08	1 9	1 8
1e	1 29	1 08	2 9	2 7
2d	1 31	1 21	0 7	0 5
2e	1 31	1 21	1 1	0 7
1j	1 39	1 09	18 0	12 5
1i	1 62	1 19	40 8	52 8
2i	1 64	1 29	3 4	1 8
1k	1 65	0 92	1 9	1 9
2k	1 68	1 08	0 4	0 3

*Retention time of acetate derivatives relative to cholesteryl acetate (1 00) on SE-30 capillary column, 250°

†Retention time of sterols relative to cholesterol (1 00) on a Partisil ODS-2 column and methanol as eluent

The ^{1}H NMR data ($CDCl_3$) for the methyl groups of the sterols from the two phanerogames and reference compounds are given below The NMR spectra were recorded at 270 MHz, unless otherwise specified

Campesterol (**1d**) ^{1}H NMR δ0 678 (3H, *s*, H-18), 0 775 (3H, *d*, J = 6 8 Hz, H-28), 0 802 (3H, *d*, J = 7 0 Hz, H-27), 0 853 (3H, *d*, J = 6 7 Hz, H-26), 0 911 (3H, *d*, J = 6 6 Hz, H-21), 1 008 (3H, *s*, H-19)

22,23-*Dihydrobrassicasterol* (**1e**) ^{1}H NMR δ0 678 (3H, *s*, H-18), 0 775 (3H, *d*, J = 6 8 Hz, H-28), 0 782 (3H, *d*, J = 6 9 Hz, H-27), 0 858 (3H, *d*, J = 7 0 Hz, H-26), 0 919 (3H, *d*, J = 6 7 Hz, H-21), 1 008 (3H, *s*, H-19)

Brassicasterol (**1g**) ^{1}H NMR (500 MHz) δ0 692 (3H, *s*, H-18), 0 818 (3H, *d*, J = 7 8 Hz, H-27), 0 832 (3H, *d*, J = 7 8 Hz, H-26), 0 911 (3H, *d*, J = 6 6 Hz, H-28), 1 008 (3H, *s*, H-19), 1 010 (3H, *d*, J = 6 7 Hz, H-21)

Epibrassicasterol (**1f**) ^{1}H NMR (500 MHz) δ0 692 (3H, *s*, H-18), 0 818 (3H, *d*, J = 7 0 Hz, H-27), 0 833 (3H, *d*, J = 7 0 Hz, H-26), 0 909 (3H, *d*, J = 6 7 Hz, H-28), 1 001 (3H, *d*, J = 6 6 Hz, H-21), 1 008 (3H, *s*, H-19)

Sitosterol (**1i**) ^{1}H NMR δ0 680 (3H, *s*, H-18), 0 814 (3H, *d*, J = 6 8 Hz, H-27), 0 835 (3H, *d*, J = 6 7 Hz, H-26), 0 846 (3H, *t*, J = 7 2 Hz, H-29), 0 921 (3H, *d*, J = 6 5 Hz, H-21), 1 009 (3H, *s*, H-19)

Clionasterol (*isolated from marine alga* Caulerpa prolifera) ^{1}H NMR δ0 680 (3H, *s*, H-18), 0 813 (3H, *d*, J = 6 8 Hz, H-27), 0 832 (3H, *d*, J = 6 8 Hz, H-26), 0 855 (3H, *t*, J = 7 4 Hz, H-29), 0 927 (3H, *d*, J = 6 5 Hz, H-21), 1 010 (3H, *s*, H-19)

Stigmasterol (**1j**) ^{1}H NMR δ0 699 (3H, *s*, H-18), 0 796 (3H, *d*, J = 6 6 Hz, H-27), 0 806 (3H, *t*, J = 7 9 Hz, H-29), 0 846 (3H, *d*, J = 6 6 Hz, H-26), 1 011 (3H, *s*, H-19), 1 022 (3H, *d*, J = 6 6 Hz, H-21)

5α-*Campestanol* (**2d**) ^{1}H NMR δ0 643 (3H, *s*, H-18), 0 769 (3H, *d*, J = 7 0 Hz, H-28), 0 795 (3H, *d*, J = 6 9 Hz, H-27), 0 795 (3H, *s*, H-19), 0 845 (3H, *d*, J = 6 8 Hz, H-26), 0 890 (3H, *d*, J = 6 6 Hz, H 21)

5α-*Ergostanol* (**2e**) ^{1}H NMR δ0 643 (3H, *s*, H-18), 0 769 (3H, *d*, J = 7 0 Hz, H 28), 0 776 (3H, *d*, J = 6 9 Hz, H-27), 0 795 (3H, *s*, H-19), 0 848 (3H, *d*, J = 6 9 Hz, H-26), 0 898 (3H, *d*, J = 6 6 Hz, H-21)

5α-*Stigmastanol* (**2i**) ^{1}H NMR δ0 648 (3H, *s*, H-18), 0 800 (3H, *s*, H-19), 0 811 (3H, *d*, J = 6 3 Hz, H-27), 0 832 (3H, *d*, J = 6 3 Hz, H-26), 0 842 (3H, *t*, J = 6 8 Hz, H-29), 0 903 (3H, *d*, J = 6 3 Hz, H-21)

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REFERENCES

1 Goad, L J and Goodwin, T W (1972) in *Progress in Phytochemistry* (Reinhold, L and Liwschitz, V, eds) Vol 3, p 113 Interscience, London
2 Nes, W R and Mc Kean, M L (1977) *Biochemistry of Steroids and Other Isoprenoids* University Park Press, Baltimore
3 Scheid, F and Benveniste, P (1979) *Phytochemistry* **18**, 1207
4 Misso, N L A and Goad, L J (1984) *Phytochemistry* **23**, 73
5 Nes, W R, Krevitz, K, Joseph, J, Nes, W D, Harris, B, Gibbons, G F and Patterson, G W (1977) *Lipids* **12**, 511
6 Rubinstein, I, Goad, L J, Clague, A D H and Mulheirn, L J (1976) *Phytochemistry* **15**, 195
7 Nes, W R, Krevitz, K and Behzadan, S (1976) *Lipids* **11**, 118
8 Wright, J L C, McInnes, A G, Shimizu, S, Smith, D G, Walter, J A, Idler, D and Khalil, W (1978) *Can J Chem* **56**, 1898
9 Chiu, P L and Patterson, G W (1981) *Lipids* **16**, 203
10 Lang, R W and Djerassi, C (1982) *Helv Chim Acta* **65**, 407
11 Schmitz, F J (1978) in *Marine Natural Products* (Scheuer, P J, ed), Vol 1, p 241 Academic Press, New York
12 Itoh, T, Sica, D and Djerassi, C (1983) *J Chem Soc Perkin Trans* 1, 147
13 Matsumoto, T, Shimizu, N, Shigemoto, T, Itoh, T, Iida, T and Nishioka, A (1983) *Phytochemistry* **22**, 789