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EFFECTIVE SEPARATION AND C-24 STEREOCHEMICAL ASSIGNMENT OF EPIMERIC 24-ISOPROPENYLCHOLESTEROLS

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Abstract: Separation of epimeric 24-isopropenylcholesterols (1a) was effectively achieved by reversed-phase HPLC. The C-24 stereochemistry of these epimers was determined on the basis of chemical and spectroscopic evidence.

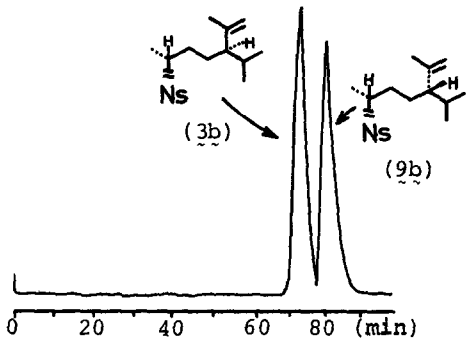
In the preceding communication,[1] we reported the effective separation of C-24 epimers of typical 24-alkylated sterol benzoates by reversed-phase high performance liquid chromatography.

Recently, isopropenylcholesterol, a non-conventional side chain sterol, has been obtained as the 24-epimeric mixture (1a) from a Caribbean sponge, *Verongia cauliformis*, by Djerassi and co-workers,[2] who also synthesized 1a from fucosterol (2) and obtained one of the C-24 epimers in an almost pure state. We also isolated 24ξ-isopropenylcholesterol (9a) from *Nervilia purpurea*, an Orchidaceous plant.[3] However, the stereochemistry at the C-24 position in this isopropenylcholesterol was uncertain. This paper describes the effective separation of 1a and the stereochemical assignment of each epimer.

Isopropenylcholesterol (1a) was prepared from fucosterol (2) according to Djerassi's description.[2] Benzoylation of 1a yielded the corresponding benzoate (1b, a mixture of C-24 epimers), which revealed two peaks (in an approximate ratio of 51:49) on HPLC with a reversed-phase column using chloroform-acetonitrile (2:8) as the eluent (Fig. 1). Each of these epimers was successfully isolated by repeated preparative HPLC; the more mobile epimer (3b) showed mp 130-131°C, $[\alpha]_D$ -15.4° ($CHCl_3$), and the less mobile one (9b) mp 150-151°C, $[\alpha]_D$-19.2° ($CHCl_3$).

Alkaline hydrolysis of each of epimeric benzoates, 3b and 9b, afforded the corresponding sterols: 3a, mp 141-142°C, $[\alpha]_D$-38.8° ($CHCl_3$), $C_{30}H_{50}O$ (M^+, 426.3910; Calcd, 426.3861), and 9a, mp 135-135.3°C, $[\alpha]_D$-39.8° ($CHCl_3$), $C_{30}H_{50}O$ (M^+, 426.3887; Calcd, 426.3861), respectively. The ^{1}H-NMR spectral data for 3a, 3b, 9a, and 9b are given in Table 1.

In order to determine the stereochemistry at the C-24 position in 3a and 9a, we then examined the transformation of 3b and 9b into the corresponding 24-



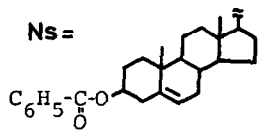


Fig. 1. HPLC chromatogram of 24-isopropenylcholesterol benzoate (1b)
Conditions: column, TSK-GEL ODS-120A (25cm x 4.6mm i.d.); solvent, $CHCl_3$-CH_3CN(2:8); flow rate, 0.6 ml/min; temperature, 20°C; detector setting, UV 240 nm.

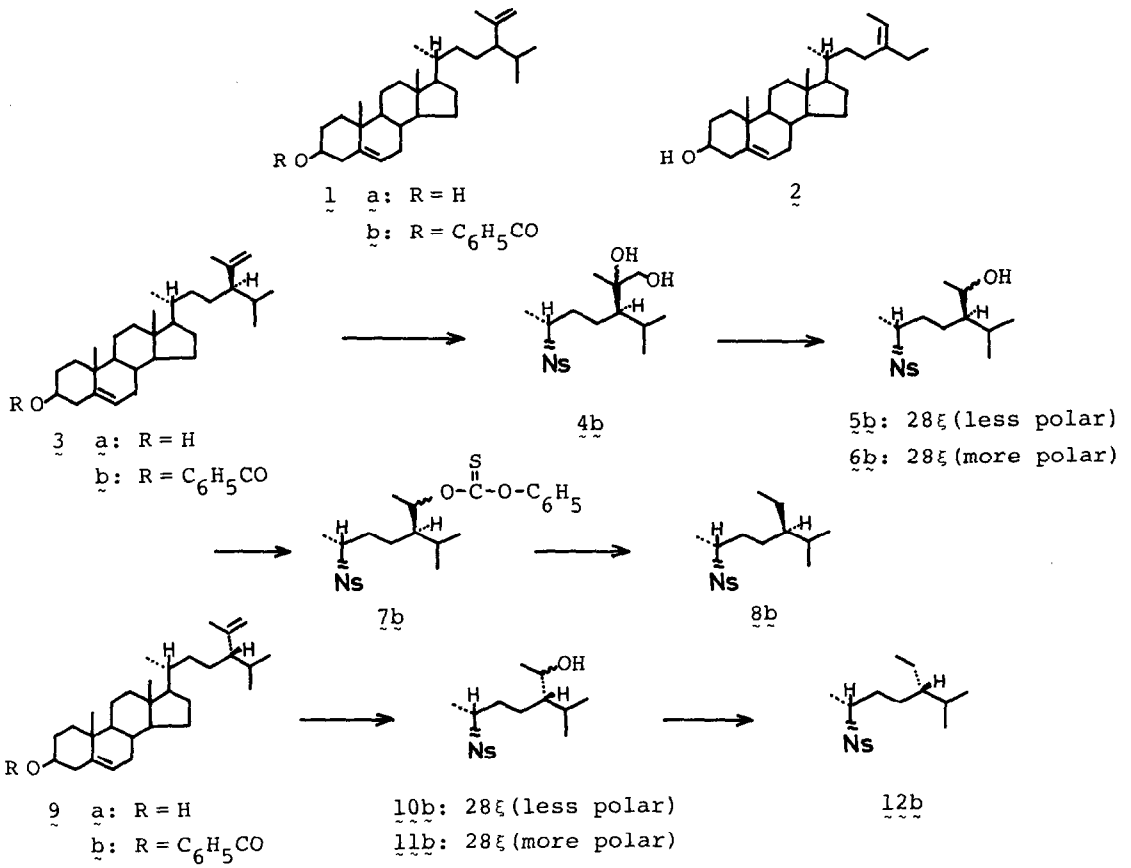


Chart 1

ethyl compounds, 8b and 12b, respectively.

Oxidation of 3b with OsO_4 in a controlled condition gave a diol (4b). Brief treatment of 4b with HIO_4 in aqueous dioxane, followed by $NaBH_4$ reduction, gave

Table 1. 200 MHz ^{1}H-NMR spectral data for synthetic 24R- and 24S-isopropenylcholesterols and natural 24S-isopropenylcholesterol from *Nervilia purpurea* and their benzoates (δ values in $CDCl_3$ and coupling constants in Hz)

Compound (C24 config.)	3-H	6-H	18-H	19-H	21-H	26-H, 27-H[1)]	29-H	30-H	Others
3a (synthetic)[2)] ($\tilde{R}/\alpha$)	3.53 m	5.36 br.d (5.4)	0.673 s	1.006 s	0.906* d (5.8)	0.798*, 0.904* d d (6.4, 6.6)	1.560 br.s	4.62, 4.74 br.s br.s	
9a (synthetic) ($\tilde{S}/\beta$)	3.52 m	5.35 br.d (5.1)	0.666 s	1.006 s	0.911* d (6.0)	0.802*, 0.922* d d (6.0, 6.6)	1.565 br.s	4.60, 4.74 br.s br.s	
9a (natural) ($\tilde{S}/\beta$)	3.52 m	5.35 br.d (5.5)	0.666 s	1.006 s	0.911* d (5.8)	0.802*, 0.922* d d (6.4, 6.2)	1.566 br.s	4.60, 4.73 br.s br.s	
3b (synthetic) ($\tilde{R}/\alpha$)	4.89 m	5.44 br.d (4.3)	0.684 s	1.067 s	0.911* d (6.0)	0.802*, 0.911* d d (6.2, 6.0)	1.563 br.s	4.63, 4.76 br.s br.s	7.47, 8.08 (phenyl) m m
9b (synthetic) ($\tilde{S}/\beta$)	4.88 m	5.44 br.d (4.3)	0.676 s	1.067 s	0.914* d (6.0)	0.804*, 0.929* d d (6.2, 6.4)	1.566 br.s	4.61, 4.75 br.s br.s	7.47, 8.08 (phenyl) m m
9b (natural) ($\tilde{S}/\beta$)	4.88 m	5.44 br.d (4.5)	0.678 s	1.068 s	0.915* d (6.0)	0.806*, 0.930* d d (6.2, 6.4)	1.569 br.s	4.61, 4.75 br.s br.s	7.47, 8.08 (phenyl) m m

* Assignments may be interchanged in each compound.

1) The higher field signal was arbitrarily assigned to the 27-methyl group.

2) The ^{1}H-NMR property of this compound is identical with that of Djerassi's synthetic 24ξ-isopropenylcholesterol obtained in almost pure state; see ref. 2.

28-epimeric alcohols, 5b and 6b, which could be separated by preparative TLC. The less polar alcohol (5b), mp 131-133°C, was converted to phenoxythiocarbonate (7b) and subsequently reduced with tributyltin hydride[4] to give a crystalline product, which showed three peaks (in an approximate ratio of 25:38:37) on HPLC. Each of these peaks could be isolated by repeated preparative HPLC; the third peak was identified as sitosterol benzoate (8b)[5] by MS and HPLC comparisons with an authentic sample, while the other peaks were assigned as elimination products on the basis of their MS data (m/z 394, $M^+-C_6H_5COOH$).[6] On the other hand, the more polar alcohol (6b), mp 136-138°C, was also deoxygenated in the same manner to give sitosterol benzoate (8b).

Similarly, the less mobile epimer (9b) was converted to epimeric 28-alcohols, 10b, mp 161-161.5°C, and 11b, mp 132-132.5°C. These compounds (10b and 11b) were subjected to the deoxygenation reaction and the separation of the products by preparative HPLC gave the same compound (12b), which was found to be identical with authentic clionasterol benzoate (12b)[5] by means of MS and HPLC.

From the foregoing evidence, the stereochemistry at the C-24 position of the more mobile epimer (3b) and the less mobile one (9b) was confirmed to be 24R and 24S, respectively.

Next, we examined the stereochemistry of 24ξ-isopropenylcholesterol (9a) isolated from *Nervilia purpurea*. Benzoylation of 9a gave the corresponding benzoate (9b), mp 151-153°C, which showed single peak on HPLC, measured under the same conditions. The ^{1}H-NMR spectra of 9a and 9b are given in Table 1. This compound was proved to be 24S-isopropenylcholesterol benzoate (9b), described above, by MS, ^{1}H-NMR, and HPLC comparisons. Therefore the stereochemistry at the C-24 position in 9a was established to be 24S configuration.

It should be mentioned here that any trace of its epimeric counterpart (3a) could not be found in *N. purpurea*. It is a particular interest from the biogenetic view point that only one of the 24-epimers exists in the plant, while in the sponge, both of the 24-epimers occur.[2]

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References and Notes

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6) The second peak was assigned as fucosterol benzoate by MS and HPLC analyses.

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