Identification of Isomers of Cholestane, $C_{27}H_{48}$

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Summary The properties of 5α-cholestane and six stereoisomers have been studied to evaluate methods of identification of steranes from geological sources.

ANALYSIS of the steranes present in crude oils and sedimentary rocks often reveals a complex mixture of isomers and homologues, predominantly of composition $C_{27}H_{48}$, $C_{28}H_{50}$, and $C_{29}H_{52}$. Identification of these components has relied heavily on combined g.l.c. and mass spectrometry (m.s.).[1] However, the structural and stereochemical information provided by m.s. is limited, giving the number of carbon atoms in the sidechain (but not its structure), the presence of extra methyl substituents in rings A or B, and the geometry of the A/B ring junction. The use of the g.l.c. parameter requires the availability, impractical for steranes, of reference samples of all isomers likely to be present. To aid the assignment of exact structures to components in geological specimens we have sought additional and more discriminating techniques, such as o.r.d. and ^1H n.m.r. spectroscopy, and have tested them on cholestane (I) and six of its stereoisomers (II)—(VII).† Results are summarised in the Table.

The mass spectra of these hydrocarbons are identical within the reproducibility of the instrument, except for those of (II) and (IV), which show enhanced peaks at *m/e* 151 and 218, respectively. The retention times vary in a complex manner. In principle, isomers (I)—(VII) can be distinguished by g.l.c.–m.s. [(I) and (III) should be resolved

TABLE

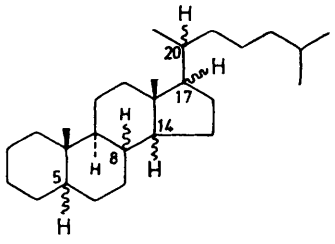
Properties of cholestane isomers

		(I)	(II)	(III)	(IV)	(V)	(VI)	(VII)
Mass spectra[a]	218/217 ..	0·56	0·40	0·56	1·10	0·55	0·46	0·48
	151/149 ..	0·17	1·07	0·14	0·12	0·21	0·34	0·15
G.l.c.[b]	SE-30[c]	1·00	0·92	0·98[e]	0·87	0·91	0·80	0·73
	Carbowax 20M[d] ..	1·00	0·89	1·01[e]	0·83	0·89	0·76	0·68
O.r.d.[f]	589 nm	+101	+104	+163	+217	+41	−11	−116
	333 nm	+399	+377	+637	+797	+182	−17	−437
	233 nm	+1310	+1030	+2120	+2170	+734	+43	−1180
^1H n.m.r.[g]	18-Me	0·645	0·641	0·888[i]	0·981	0·643	0·739	0·742
($CDCl_3$)	19-Me	0·775	0·915	0·848[i]	0·753	0·774	0·778	0·766
	21-Me[h]	0·897	0·899	0·90	0·835	0·802	0·807	0·853
	26,27-Me_2[h] ..	0·861	0·863	0·861	0·865	0·862	0·860	0·873
(C_6D_6)	18-Me	0·690	0·677	0·908[i]	1·070	0·694	8·818[i]	0·809[i]
	19-Me	0·795	0·962	0·885[i]	0·771	0·789	0·789[i]	0·782[i]
	21-Me[h]	1·015	1·021	1·014	0·948	0·913	0·936[j]	0·973[j]
	26,27-Me_2[h] ..	0·927	0·933	0·917	0·919	0·933	0·915	0·908

[a] Peak height ratios, AEI MS-30 instrument, g.l.c. inlet, 70 eV. [b] Retention times relative to (I); cholestane internal standard except for (III). [c] 6 ft × ⅛in, 5% SE-30, 250° (cholestane = 17 min). [d] 10 ft × ¼ in, 10% Carbowax 20M, 250° (cholestane = 45 min). [e] 5β-Cholestane internal standard. [f] n-Hexane solutions, 20°, conc. 0·2—0·8% ([Φ]/degree). [g] 100 MHz spectra, conc. 0·3—1·7% (δ, p.p.m. from Me_4Si). [h] Doublets, *J ca.* 6 Hz. [i] These assignments may be reversed. [j] Approximate values.

† The stereochemistry of cholestane is here defined as that shown in formula (I), and only deviations from this are indicated when referring to other isomers. Details of synthesis will be published elsewhere.

by capillary g.l.c., and a mixture of (II) and (V), even if not resolved, should be amenable to m.s. analysis]. However, the g.l.c. and m.s. properties of other isomers, still unavailable, cannot be predicted from the present data and exact coincidence of these properties for two or more isomers cannot be ruled out. Therefore, the identification by g.l.c.–m.s. of the component(s) of a sterane peak from a crude oil or rock extract remains uncertain.



	5-H	8-H	14-H	17-H	20-H
(I)	α	β	α	α	β (20*R*)
(II)	β	β	α	α	β (20*R*)
(III)	α	α	β	α	β (20*R*)
(IV)	α	β	β	α	β (20*R*)
(V)	α	β	α	α	α (20*S*)
(VI)	α	β	α	β	α (20*S*)
(VII)	α	β	α	β	β (20*R*)

In contrast, o.r.d. and ^{1}H n.m.r. spectra of the isomers show differences of considerable diagnostic value. Stereochemical changes at ring junctions cause the plain positive o.r.d. curve of cholestane (I) to be retained or enhanced (II)—(IV); inversion of configuration at C-20 lowers rotations considerably (V), while the 17β-H epimers have negative rotations (VI)—(VII). The marked effect of changes in substitution and stereochemistry around ring D is supported by the o.r.d. spectra of 5α-androstane and 5α-pregnane, which have small positive rotations. The ^{1}H n.m.r. spectra of the cholestane isomers show marked variations of the methyl group chemical shifts with changes in stereochemistry. In (II) and (IV) angular methyl groups, eclipsed by 5β- and 14β-hydrogens, show characteristic deshielding. Sidechain isomers (V)—(VII) exhibit differences in the 18- and 21-methyl signals. In addition, signals overlapping in $CDCl_3$ or C_6D_6 are often resolved by a change to the alternate solvent. The solvent shift [$\delta(CDCl_3) - \delta(C_6D_6)$], can also be used to advantage in identification.

The value of ^{1}H n.m.r. and o.r.d. spectroscopy in identifying the 24-alkylcholestanes has been demonstrated earlier,[2] and with the extension of these techniques to the compounds reported here, a more rigorous approach can be made to the structural analysis of steranes in geological samples. Although the individual components must be isolated, *e.g.*, by preparative g.l.c., the quantities required are small (*ca.* 1—5 mg). Where preparative g.l.c., even combined with other methods such as thiourea adduction and adsorption chromatography, fails to separate isomer mixtures, ^{1}H n.m.r. analysis can give structural information sufficient to guide the synthesis of the reference hydrocarbons required for unambiguous identification of the components.

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