Tetrahedron Letters,Vol.24,No.33,pp 3461-3464,1983 0040-4039/83 $3.00 + .00
Printed in Great Britain

CHEMICAL PROOF OF ABSOLUTE CONFIGURATION OF 24,26-CYCLOCHOLESTEROL BY BIOMIMETIC CONVERSION TO 27-NORERGOSTENES.

Cesar A. N. Catalan[1] and Carl Djerassi*

Department of Chemistry, Stanford University, Stanford, California 94305, USA

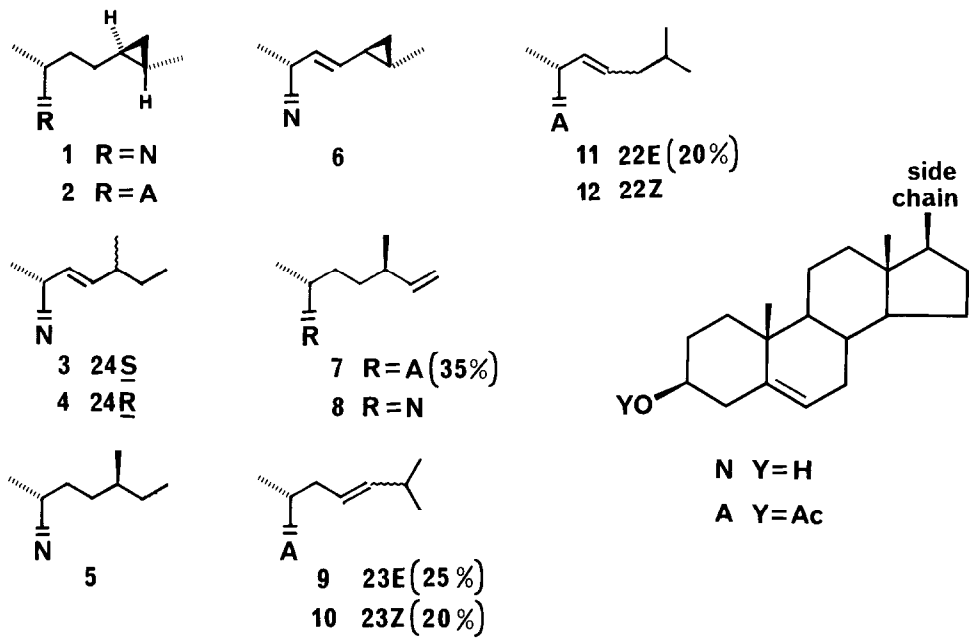
Abstract: Acid-catalyzed isomerization of 24,26-cyclocholesterol acetate gave (24R)-24-methyl-27-nor-5,25-cholestadien-3β-ol acetate, thus establishing the absolute configuration of the cyclopropyl sterol and providing a biomimetic route to the 27-norergostene type marine sterols.

Recently we isolated[2,3] from marine sources the novel cyclopropyl sterols 24,26-cyclocholesterol (1) and papakusterol (6) which contain a cyclopropane ring within the cholesterol side chain. Both 1 and 6 are unusual in that no bioalkylation is involved in their formation in contrast to all other marine cyclopropyl sterols[4] where non-squalenoid carbons are implicated. Another biosynthetically interesting aspect of the structure of 1 and 6 is that fission of the C-25, C-26 bond provides the 27-norergostene side chain[5,6] (e.g., 3, 4, 5) which has only been encountered among marine organisms and whose biosynthetic origin is still obscure.

Although the trans disposition of the substituents of the cyclopropane ring of 1[2] had been established, its absolute configuration was not elucidated. Such stereochemical knowledge is indispensable in order to synthesize the appropiate biosynthetic precursors for eventual labelling and incorporation studies. This fact together with our finding that acid-catalyzed isomerization of certain asymmetrically substituted cyclopropanes proceeds regiospecifically and stereospecifically[7,8] prompted us to use this reaction for determining the absolute configuration of 1 since one of the possible ring-opening products could be an occelasterol (3) or epi-occelasterol (4) analog.

Treatment of 24,26-cyclocholesterol acetate (2) (5 mg) with a 5% solution of trifluoroacetic acid in anhydrous benzene (48 hr, r.t.) yielded a mixture free of starting material. Separation by reverse-phase HPLC (two Altex Ultrasphere 5μ ODS columns in series with methanol as eluent at 3.0 ml/min) yielded six short retention time fractions consisting of trifluoroacetates (70% of the mixture; RT 32 to 39 min) and three fractions containing the isomerization products (30% of the mixture).

Fraction 7 (RT 67 min) representing 35% of the isomerization products contained a single component. Its ^{1}H-NMR spectrum displayed the characteristic signals for a terminal double bond (see Table) and two methyl doublets in addition to the singlets corresponding to C-18 and C-19 and the acetate grouping which indicated it to be a 24-methyl-27-nor-5,25-cholestadien-



3β-ol acetate (7). The NMR spectrum of the free sterol 8 in C_6D_6 showed the C-18 methyl signal at 0.651 ppm typical of a 24R-methyl substituent.[9] Further confirmation of the absolute configuration at C-24 was obtained by selective hydrogenation of the Δ^{25}-double bond of 8 with $(Ph_3P)_3Rh(I)Cl$ in C_6H_6 (3 hr, r.t.) to give the known (24S)-24-methyl-27-norcholest-5-en-3β-ol (5).[6,10] Since we have established[3] by synthesis the trans arrangement of the substituent on the cyclopropane ring in papakusterol (6)[11] which in turn was related[2] to 1, the absolute configuration for both sterols is 24S,25S.

Fraction 8 (RT 69 min) provided a 5:4 mixture of (23E) and (23Z)-23-dehydrocholesterol acetates (9) and (10), respectively. They were separated by reverse-phase HPLC using Altex columns and acetonitrile-methanol-ethyl acetate 22:9:9 at 3.0 ml/min as eluent (23Z isomer: 54.3 min; 23E isomer: 55.8 min) and characterized by their 360 MHz ^{1}H-NMR data (Table I) and comparison with authentic specimens.[12] Finally, fraction 9 (RT 72 min) was identified as (22E)-22-dehydrocholesterol acetate (11) free of the corresponding 22Z isomer (12).[12]

The ring opening of (24S,25S)-24,26-cyclocholesterol acetate (2) to yield the occelasterol-type[5] sterol 7 suggests a new potential pathway for the biosynthesis of 27-nor-sterols since 1 as well as papakusterol (6) give rise to compounds with the same configuration at C-24 as those found in naturally occurring 24-methyl-27-nor sterols.[5]

TABLE I. Selected 360 MHz ^{1}H-NMR chemical shifts for (24S,25S)-24,26-cyclocholesterol (1), its isomerization products and related compounds.

Compound	C-18	C-19	C-21	C-26	C-27	C-28	C-24	C-25	others
1[a]	0.677	1.006	0.886 (d, 6.6)	0.10 (2H, m)	0.998 (d, 5.9)	---	0.28 (m)	0.39 (m)	
2[a]	0.676	1.017	0.887 (d, 6.6)	0.10 (2H, m)	0.999 (d, 6.0)	---	0.29 (m)	0.40 (m)	Ac= 2.031
7[a]	0.666	1.013	0.907 (d, 6.5)	4.931[d] 4.887[e]	---	0.966 (d, 6.7)		5.703[f]	Ac= 2.034
8[b]	0.651	0.942	0.977[c] (d, 6.6)	5.028[d] 4.992[e]	---	1.012[c] (d, 6.7)		5.738[f]	
5[a]	0.676	1.007	0.906 (d, 6.6)	0.855 (t, 7.3)	---	0.826 (d, 6.3)			
9[a]	0.677	1.014	0.895 (d, 6.6)	0.964 (d, 6.7)	0.964 (d, 6.7)	---			23-H, 24-H, 6-H=5.3-5.4(m)
10[a]	0.684	1.017	0.915[c] (d, 6.6)	0.930[c] (d, 6.6)	0.932[c] (d, 6.7)	---			23-H, 24-H= 5.18-5.28(m) Ac= 2.031
10[b]	0.631	0.914	1.005[c] (d, 6.3)	1.005[c] (d, 6.3)	1.021[c] (d, 6.4)	---			23-H, 24-H, 6-H=5.3-5.4(m) Ac= 1.747
11[a]	0.691	1.019	1.007 (d, 6.5)	0.858 (d, 6.6)	0.860 (d, 6.6)	---			22-H= 5.204[g] 23-H= 5.281[h] Ac= 2.033

[a] In $CDCl_3$; [b] In C_6D_6; [c] These assignments could be reversed; [d] ddd, 17.6, 1.6 and 1.1 Hz; [e] ddd, 10.2, 1.1 and 0.6 Hz; [f] ddd, 17.5, 10.3 and 7.4 Hz; [g] dd, 15.2 and 7.7 Hz; [h] ddd, 15.2, 6.8 and 6.8 Hz.

ACKNOWLEDGEMENTS

Financial support was provided by NIH Grants No. GM-06840 and No. GM-28352. C. A. N. C. thanks the Consejo Nacional de Investigaciones Cientificas y Tecnicas de la Republica Argentina for a postdoctoral fellowship at Stanford University. Use of the NMR facilities at Stanford Nuclear Magnetic Resonance Laboratory was funded by NSF grant No. GP-23633 and NIH grant No. RR-00711.

REFERENCES AND NOTES

1. Fellow of the Consejo Nacional de Investigaciones Cientificas y Tecnicas de la Republica Argentina while on leave from the Universidad Nacional de Tucuman, Argentina.
2. C. A. N. Catalan, V. Lakshmi, F. J. Schmitz and C. Djerassi, Steroids 40, 455 (1982).
3. C. Bonini, R. B. Kinnel, M. Li, P. J. Scheuer and C. Djerassi, Tetrahedron Lett. 24, 277 (1983).
4. For leading references see C. Djerassi, Pure & Appl. Chem. 53, 873 (1981); F. J. Schmitz in Marine Natural Products (P. J. Scheuer, ed.), Academic Press, N.Y. 1978, chapter 5.
5. M. Kobayashi and H. Mitsuhashi, Tetrahedron 30, 2147 (1974); idem, Steroids 24, 399 (1974) and 26, 605 (1975).
6. Y. Hirano and C. Djerassi, J. Org. Chem. 47, 2420 (1982). The ^{1}H-NMR data for the C-26 and C-28 methyl groups of 5 were reported erroneously in this publication; the correct data are reported here in Table I.
7. R. W. Lang and C. Djerassi, Helv. Chim. Acta 65, 407 (1982); idem, Tetrahedron Lett. 23, 2063 (1982).
8. J. Proudfoot and C. Djerassi, to be published; M. Zimmerman and C. Djerassi, to be published.
9. B. V. Crist, C. A. N. Catalan and C. Djerassi, Steroids, in preparation.
10. It should be noted that applying the Kahn-Prelog-Ingold rules, hydrogenation of the 24R epimer 9 gives the 24S sterol 5 owing to an inversion in the priority order of the substituents.
11. Natural papakusterol (6) is a mixture of both isomers with predominance of the 24S,25S diastereoisomer (see ref. 3). We isolated a specimen of 6, mp 138-140°C from an as yet unclassified species of Pseudothesis --a "deep-sea" gorgonian-- provided by Prof. Paul J. Scheuer of the University of Hawaii. Its 360 MHz ^{1}H-NMR spectrum indicated that it consisted of 85% (24S,25S)-papakusterol (6) and 15% of the 24R,25R isomer.
12. R. W. Lang and C. Djerassi, J. Org. Chem. 47, 625 (1982).

(Received in USA 11 May 1983)