Tetrahedron Vol. 42, No. 17, pp 4843 to 4847, 1986
Printed in Great Britain.

0040-4020/86 $3.00 + .00

CONFIGURATION ASSIGNMENT OF 24R- AND 24S-ISOMERS OF 29-OXYGENATED STEROIDS BY ^{1}H AND ^{13}C NMR SPECTROSCOPY

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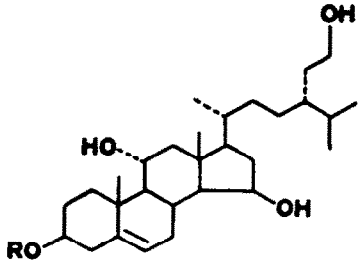
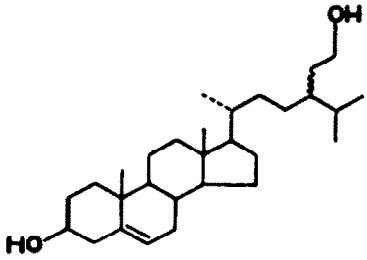
(*Received in UK 20 June* 1986)

Abstract- Epimeric 24-hydroxyethyl and 24-carboxymethyl steroids were synthesized, their ^{1}H and ^{13}C-NMR spectra recorded and the signals assigned. Based on those assignements information regarding the stereochemistry of the C-24 carbon of the steroids under study was obtained.

Since the isolation of the oogoniols (1) from the water mold Achlya[1] and the elucidation of their structure, a number of marine steroid glycosides have been isolated with the aglycone moiety containing a hydroxyethyl substituent at position 24 of the cholesterol side chain[2,3]. To determine the structure of these steroids it was necessary to interpret spectral data and to effect some chemical transformations. In particular in order to assign the 24-configuration of the 24-hydroxyethyl substituent of the oogoniols by ^{1}H NMR spectroscopy the synthesis of the model epimers 29-hydroxyclionasterol (2a) and 29-hydroxysitosterol (2b) was necessary so as to compare their ^{1}H NMR spectra with those of the oogoniols.[4,5]



1



2

R = $(CH_3)_2CHCO$, CH_3CH_2CO, CH_3CO, H a : 24R; b : 24S

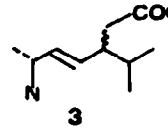
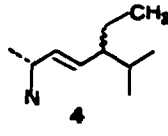
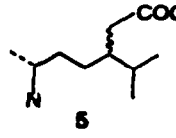
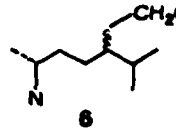
Moreover 24-hydroxyethyl substituted steroids were required as key intermediates in synthetic studies on oogoniols[6] and on plant growth promoter brassinolides.[7] Since we observed that ^{1}H NMR alone did not permit an unambiguous differentiation between 24R and 24S epimers we undertook a systematic investigation on the ^{1}H and ^{13}C NMR spectroscopy of a series of 24R- and 24S-hydroxyethyl and carboxymethyl steroids (3-6) regiospecifically and stereospecifically synthesized. Our aim was to indicate which technique would be of the greatest diagnostic utility to define the configuration at this position in unknown steroids with the same side chain. This paper

reports the results of this study.

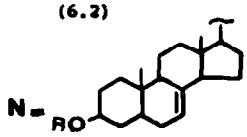
RESULTS AND DISCUSSION

^{1}H NMR Studies: The chemical shifts of the methyl protons of C-24 epimeric pairs of steroids (3-6) having at C-24 a carboxymethyl or an hydroxyethyl substituent are listed in Table 1.

Table 1. 250 MHz ^{1}H NMR data of (24R)- and (24S)-24-substituted sterols[a]

Sterol and Stereochemistry		δ C-26,27	Δδ C-26,27	δ C-21	δ C-19	δ C-18
 3	a, 24R	d 0.83, d 0.87 (6.9, 6.5)	0.04	d 1.01 (6.6)	s 0.81	s 0.53
	b, 24S	d 0.85, d 0.88 (6.9, 6.5)	0.03	d 0.99 (6.5)	s 0.81	s 0.53
 4	a, 24R	d 0.83, d 0.88 (7.0, 6.5)	0.05	d 1.04 (6.8)	s 0.81	s 0.56
	b, 24S	d 0.86, d 0.88 (7.0, 6.5)	0.02	d 1.03 (6.1)	s 0.81	s 0.56
 5	a, 24R	d 0.82, d 0.88 (7.0, 6.5)	0.06	d 0.92 (6.1)	s 0.81	s 0.53
	b, 24S	d 0.85, d 0.86 (6.9, 6.5)	0.01	d 0.92 (6.1)	s 0.81	s 0.53
 6	a, 24R	d 0.83, d 0.86 (7.0, 6.5)	0.03	d 0.93 (6.2)	s 0.80	s 0.54
	b, 24S	d 0.85, d 0.86 (6.9, 6.5)	0.01	d 0.93 (6.2)	s 0.80	s 0.54

[a] Coupling constants (in parentheses) are in Hz. Differences in chemical shifts (Δδ) for C-26,27 methyl protons are in ppm.

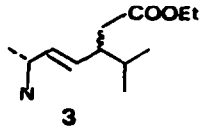
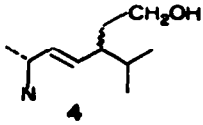
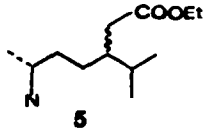
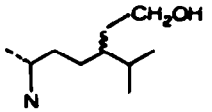


Each epimeric couple of oxygenated compounds (3-6) showed identical chemical shifts for C-18, C-19 and C-21 methyl signals (the C-21 methyl signal being different only in the case of the epimeric couples (3 and 4) with an unsaturated side chain). However the differentiation of the couples (3-6) appeared possible on the basis of a different chemical shift difference (Δδ) observed between the C-26 and C-27 methyl signals in the 24R- and 24S-isomers. The observed values show that in the case of 24R carboxymethyl and hydroxyethyl isomers such a difference ranges between 0.06 and 0.03 ppm, while in the case of the 24S-isomers the difference is always under 0.03 ppm. In addition, in the case of the 24R-epimer the doublet of the less deshielded isopropyl methyl group is constantly centered at higher field than the corresponding methyl group of the 24S epimer (Δδ = 0.02-0.03 ppm). Such behaviour is also observed to a minor extent (Δδ = 0.01 ppm) for the same methyl groups of chondrillasterol and spinasterol[8] having a simple ethyl group at C-24. These data show that in the 24-ethyl substituted steroids a 29-oxygenated function enhances the magnetic non equivalence of the C-26 and C-27 methyl protons and chemical shift differences provide a method of distinguishing between 24-epimers, or of estimating the composition of epimeric mixtures. But a rigorous assignment of the C-24 configuration is possible only in the presence of both epimers.

^{13}C NMR Studies: The assignment of the chemical shifts to the carbons of $\Delta^{7,22}$ compounds was facilitated using (22E,24R)- and (22E,24S)-5α-ergosta-7,22-dien-3β-ols as model compounds while resonances for the alkyl-side chain carbons of Δ^7-sterols were assigned by direct comparison with the data reported for clionasterol and sitosterol.[9] The couples of the 24R- and 24S-epimers showed

strikingly similar chemical shifts for all carbons C-1 to C-21 of the nucleus and for those of the side chain (see experimental), apart from the signals attributed to the C-26, and C-27 carbon resonances reported in Table 2.

Table 2. Carbon chemical shifts of (24R)- and (24S)-24-substituted sterols in ppm

Sterol and Stereochemistry		δ C-26,27	Δδ C-26,27
 3	a, 24R	18.8, 20.4	1.6
	b, 24S	19.0, 20.5	1.5
 4	a, 24R	19.0, 20.7	1.7
	b, 24S	19.1, 20.7	1.6
 5	a, 24R	18.6, 19.7	1.1
	b, 24S	19.2, 19.3	0.1
 6	a, 24R	18.3, 19.7	1.4
	b, 24S	18.9, 19.3	0.4

Examination of the obtained resonances shows that in the presence of a C-29 oxygenated function the difference in the chemical shifts of the isopropyl methyl carbons of the steroids with a saturated side chain offers an useful information on C-24 stereochemistry. In fact the difference in chemical shift (Δδ) observed between the C-26 and C-27 carbon resonances ranges between 1.1-1.4 ppm in the case of 24-R isomers, while it ranges between 0.1 and 0.4 ppm for 24S-isomers. Thus ^{13}C NMR spectroscopy permits an unambiguous assignment of the C-24 stereochemistry of 29-oxygenated steroids with a saturated side chain. This is possible even in the presence of a single epimer. In the case of Δ^{22} unsaturated steroids the chemical shift difference between the same carbon signals is too small and the ^{13}C NMR fails to differentiate 24R- and 24S-isomers. However the assignment is still possible examining the spectrum of the compound deriving from a simple reduction of the Δ^{22} double bond.

In conclusion the ^{1}H NMR spectroscopy allows the differentiation between couples of epimers but only when comparing the spectra of both compounds. In fact the differences in the positions of the C-26 and C-27 methyl doublets even if small, when compared to the invariant C-18 and C-19 methyl singlets, give rise to significantly different peak patterns. ^{13}C NMR spectroscopy on the contrary permits an unambiguous assignment of the 24 stereochemistry and does not require the presence of both epimers when the sterol possess a saturated side chain.

EXPERIMENTAL

NMR spectra: spectra (^{1}H and ^{13}C) were recorded with a Brüker WM-250 Furier Transform Spectromer. All samples (3 to 10 mg) were dissolved in ca 0.4 ml of $CDCl_3$ and analysed in 5 mm o.d. tubes at probe ambient temperature of 27°C. The ^{13}C NMR spectra were recorded at 62.9 MHz, spectral width 15000 Hz, first in broad band ^{1}H decoupling mode. The degree of substitution of each carbon was then determined by DEPT pulse sequence experiments[10] using a polarization transfer pulses of 90° and 135° obtaining in the first case only signals for CH groups and, in the other case, positive signals for CH and CH_3 and negative ones for CH_2 groups. Polarization transfer delays were adjusted to an average CH coupling of 135 Hz. Mass spectra were recorded on a Varian 112-S by

direct inlet. TLC was performed on precoated silica gel G plates (E. Merck, HF_{254}), visualized by spraying with 70% sulfuric acid followed by heating. GLC analyses were made using a Carlo Erba Fractovap 2400 T unit using either 3% OV-17 or 1% SE-30 on Gas Chrom Q (100–120 mesh) columns operating at 220–240°C. All compounds gave satisfactory (± 0.2%) elemental analyses.

(22E,24R)-5α-Ergosta-7,22-dien-3β-ol (m.p. 174–175°C) was obtained from ergosterol[11]. (22E,24S)-5α-Ergosta-7,22-dien-3β-ol (m.p. 159–160°C; ^{13}C NMR (identical for the 24R- and 24S isomer): 37.1 (C-1), 31.4 (C-2), 70.8 (C-3), 37.9 (C-4), 40.2 (C-5), 29.6 (C-6), 117.1 (C-7), 139.0 (C-8), 49.9 (C-9), 34.1 (C-10), 21.5 (C-11), 39.4 (C-12), 43.1 (C-13), 55.0 (C-14), 22.9 (C-15), 28.1 (C-16) 55.8 (C-17), 12.1 (C-18), 13.0 (C-19), 40.4 (C-20), 21.1 (C-21), 135.2 (C-22), 131.4 (C-23), 42.7 (C-24), 33.0 (C-25), 17.6 (C-28).
(22E,24R)- and (22E,24S)-3-acetoxy-5-stigmasta-7,22-dien-29-oic acid ethyl esters (3a, m.p. 125–126°C; and 3b, m.p. 118–120°C; ^{13}C NMR (identical for 3a and 3b): 37.0 (C-1), 27.6 (C-2), 73.5 (C-3), 34.0 (C-4), 40.3 (C-5), 29.7 (C-6), 117.4 (C-7), 139.5 (C-8), 49.5 (C-9), 34.4 (C-10), 21.6 (C-11), 39.6 (C-12), 43.4 (C-13), 55.2 (C-14), 23.0 (C-15), 28.0 (C-16), 56.0 (C-17), 12.1 (C-18), 12.8 (C-19), 40.3 (C-20), 21.0 (C-21), 138.6 (C-22), 127.9 (C-23), 45.5 (C-24), 31.8 (C-25), 38.5 (C-28), 172.9 (C-29), 60.0 (CH_2O), 14.2 (CH_3), 21.2 (Ac), 170.4 (CO), were prepared according to Anastasia and Fiecchi[12].

(22E,24R)-3β,29-Dihydroxy-5α-stigmasta-7,22-diene (4a). The 24R-ester (3a) (200 mg; 0.39 mM) was dissolved in anhydrous diethyl ether (15 ml) and lithium aluminium hydride (300 mg) was slowly added. After stirring at 25°C for 6 hr, the mixture was cooled to 0°C and ice was cautiously added to destroy the unreacted hydride. Usual work-up afforded a residue (200 mg) which was crystallized from methanol to give compound (4a) (170 mg), m.p. 194–195°C. $[\alpha]_D^{20}$ 0°. IR cm^{-1}: 3600. MS: 428 (23%, M), 413 (10%, M-CH_3), 273 (42%, M-SC), 271 (100%), 255 (45%, M-SC-H_2O), 229 (37%).
^{13}C NMR: 37.3 (C-1), 31.6 (C-2), 71.1 (C-3), 38.1 (C-4), 40.4 (C-5), 29.7 (C-6), 117.6 (C-7), 139.6 (C-8), 49.6 (C-9), 34.4 (C-10), 21.6 (C-11), 39.6 (C-12), 43.4 (C-13), 55.2 (C-14), 23.1 (C-15), 28.4 (C-16) 55.9 (C-17), 12.1 (C-18), 13.0 (C-19), 40.7 (C-20), 21.2 (C-21), 138.6 (C-22), 129.4 (C-23), 46.4 (C-24), 32.4 (C-25), 36.6 (C-28), 62.0 (C-29).

(22E,24S)-3β,29-Dihydroxy-5α-stigmasta-7,22-diene (4b). The 24S-ester (3b) (200 mg; 0.39 mM) was reduced with lithium aluminium hydride (200 mg) as described for the 24R-isomer to yield, after crystallization from ethyl acetate, compound 5b (175 mg), m.p. 179–181°C. $[\alpha]_D^{20}$ -1°. IR cm^{-1}: 3600. MS: 428 (21%, M), 413 (13%, M-CH_3), 273 (32%, M-SC), 271 (100%), 255 (42%, M-SC-H_2O), 229 (37%). ^{13}C NMR: The values were identical to those reported for (4a) apart from those reported in Table 2.

24R-3β-Acetoxy-5α-stigmast-7-en-29-oic Acid Ethyl Ester (5a). The 24R-ester (3a) (200 mg; 0.39 mM), dissolved in freshly distilled ethyl acetate (20 ml), was stirred with Nickel-Raney (200 mg) in a hydrogen atmosphere at room temperature and pressure for 1 hr. Filtration and removal of the solvent afforded compound (5a) (190 mg), m.p. 88–90°C from methanol. $[\alpha]_D^{20}$ 5°. IR cm^{-1}: 1735. MS: 514 (31%, M), 499 (14%, M-CH_3), 454 (82%, M-AcOH), 439 (36%, M-CH_3-AcOH), 315 (23%, M-SC), 255 (97%, M-SC-AcOH), 213 (100%). ^{13}C NMR: 37.0 (C-1), 27.6 (C-2), 73.5 (C-3), 34.0 (C-4), 40.3 (C-5), 29.7 (C-6), 117.4 (C-7), 139.6 (C-8), 49.5 (C-9), 34.4 (C-10), 21.6 (C-11), 39.7 (C-12), 43.5 (C-13), 55.1 (C-14), 23.0 (C-15), 27.9 (C-16) 56.3 (C-17), 11.9 (C-18), 12.9 (C-19), 36.5 (C-20), 18.9 (C-21), 33.5 (C-22), 27.9 (C-23), 41.5 (C-24), 29.9 (C-25), 36.4 (C-28), 172.9 (C-29), 60.0 (CH_2O), 14.3 (CH_3), 21.3 (Ac), 170.5 (CO).

24S-3β-Acetoxy-5α-stigmast-7-en-29-oic Acid Ethyl Ester (5b). The 24S-ester (3b) (200 mg; 0.39 mM) was hydrogenated as its 24R-isomer to give compound (5b) (190 mg), m.p. 91–92°C from methanol. $[\alpha]_D^{20}$ 3°. IR cm^{-1}: 1735. MS: 514 (26%, M), 499 (14%, M-CH_3), 454 (80%, M-AcOH), 439 (36%, M-CH_3-AcOH), 315 (30%, M-SC), 255 (95%, M-SC-AcOH), 213 (100%). ^{13}C NMR: The values were identical to those reported for (5a) apart from those reported in Table 2.

24R-3β,29-Dihydroxy-5α-stigmast-7-ene (6a). The 24R-ester (5a) (200 mg; 0.39 mM) was dissolved in anhydrous diethyl ether (15 ml) and lithium aluminium hydride (300 mg) was slowly added. After stirring at 25° C for 6 hr, the mixture was cooled to 0° C and ice was cautiously added to destroy the unreacted hydride. The mixture was poured into a saturated NaCl solution and thoroughly extracted with ethyl acetate. The combined extracts were dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure to yield a white residue (200 mg) which was crystallized from ethyl acetate to give compound (6a) (175 mg), m.p. 175–176°C. $[\alpha]_D^{20}$ 0°. IR cm^{-1}: 3600. MS: 430 (100%, M), 415 (34%, M-CH_3), 412 (8%, M-H_2O), 397 (8%, M-CH_3-H_2O), 273 (28%, M-SC), 255 (70%, M-SC-H_2O), 231 (29%), 213 (30%). ^{13}C NMR: 37.4 (C-1), 31.7 (C-2), 71.1 (C-3), 38.2 (C-4), 40.5 (C-5), 29.8 (C-6), 117.6 (C-7), 139.6 (C-8), 49.7 (C-9), 34.4 (C-10), 21.7 (C-11), 39.8 (C-12), 43.5 (C-13), 55.2 (C-14), 23.0 (C-15), 28.0 (C-16) 56.3 (C-17), 11.9 (C-18), 13.0 (C-19), 36.7 (C-20), 19.0 (C-21), 34.0 (C-22), 27.6 (C-23), 41.1 (C-24), 29.7 (C-25), 34.3 (C-28), 62.2 (C-29).

24S-3β,29-Dihydroxy-5α-stigmast-7-ene (6b). The 24S-ester (5b) (200 mg; 0.39 mM) was reduced with lithium aluminium hydride as described for its 24R-isomer (see above) to yield a white residue (200 mg) which was crystallized from ethyl acetate to give compound (6b) (185 mg) m.p. 167–169°C. $[\alpha]_D^{20}$ -1°. IR cm^{-1}: 3600. MS: 430 (100%, M), 415 (32%, M-CH_3), 412 (10%, M-H_2O), 397 (8%, M-CH_3-H_2O), 273 (30%, M-SC), 255 (70%, M-SC-H_2O), 231 (32%), 213 (30%). ^{13}C NMR: The values were identical to those reported for (6a) apart from those reported in Table 2.

Acknowledgements
This research was supported by the Ministero della Pubblica Istruzione (MPI). We thank Professor Alberto Fiecchi for helpful discussions.

REFERENCES

1. T. C. McMorris, P. H. Le, M. W. Preus, S. R. Schow and G. R. Weibe, J. Org. Chem. 48, 3370 (1983), and references cited therein.
2. L. Minale, C. Pizza, A. Plomitallo, R. Riccio, F. Zollo and F. A. Mellon, Gazz. Chim. Ital. 114, 143 (1984).
3. M. Iorizzi, L. Minale, R. Riccio, M. Debray and J. L. Menou, J. Natur. Prod. 49, (1986) "in press" and references there cited.
4. M. W. Preus and T. C. McMorris, J. Am. Chem. Soc. 101, 3066 (1979).
5. J. R. Wiersig, N. Waespe-Sarcevic and C. D. Djerassi, J. Org. Chem. 44, 3374 (1979).
6. M. Anastasia, A. Fiecchi and A. Scala, J. Chem. Soc., Chem. Commun. 858 (1979).
7. J. R. Donaubauer, A. M. Greaves and T. C. McMorris, J. Org. Chem. 49, 2834 (1984).
8. W. Sucrow, M. Slopianka and H. W. Kiercher, Phytochemistry 15, 1533 (1976).
9. J. L. C. Wright, A. G. McInnes, S. Shimizu, D. G. Smith and J. A. Walter, Can. J. Chem. 56, 1898 (1978).
10. M. R. Bendall, D. T. Pegg, D. M. Doddrell and D. H. Williams, J. Org. Chem. 47, 3021 (1982).
11. W. Tadros and A. L. Bauls, Helv. Chim. Acta 58, 668 (1975).
12. M. Anastasia and A. Fiecchi, J. Org. Chem. 46, 1726 (1981).