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# CONFIGURATIONAL ASSIGNMENT OF EPIMERIC 22,23-EPOXIDES OF STEROIDS BY CARBON-13 NMR SPECTROSCOPY

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**Abstract**—The $^{13}$C-NMR spectra of epimeric 22,23-epoxides of steroids were recorded and the signals assigned. Based on these assignments information regarding the stereochemistry of the oxirane ring and conformations of the side chains of the steroids under study was obtained.

Since the discovery that brassinolide (**1**) has the remarkable biological activity to promote division and elongation of plant cells,[1] much effort has been devoted to the isolation of related natural products[2] and to the synthesis of **1** and its analogs.[3,4] Amongst the analogs, 28-homobrassinolide (**2**) is particularly interesting because it possesses biological activity and can be prepared from the readily available stigmasterol (**3**). For the synthesis of these steroids the introduction of OH groups at C-22 and C-23, with the required stereochemistry, is of great importance. In some synthetic sequences[4–8] the corresponding epoxides are crucial intermediates and we were interested in finding out a simple spectroscopic method for the unequivocal determination of their configurations. Consequently, and in view of the striking sensitivity of C shifts to steric effects that in cyclic systems allow the determination of the configuration of epoxides,[9–11] and since there is a somewhat restricted rotation of the steroidal side chain, we decided to carry out an analysis of some representative C-22, C-23 epoxides by $^{13}$C-NMR spectroscopy, as an extension of the approach used in cyclic systems.

## RESULTS AND DISCUSSION

We have found, in agreement with previous observations[12] that the C-22 and C-23 of the *R,R*-epoxide **4** show very similar chemical shifts ($\Delta\delta = 0.1$ ppm) whereas in the *S,S*-epoxide **5** those carbons are clearly distinguishable ($\Delta\delta = 4.6$ ppm). Carbon-21 show very similar chemical shifts in both compounds while an upfield shift of *ca* 2.5 ppm can be observed for C-17 of **4** in comparison with the same carbon of **5**. These differences in chemical shifts are also observed for **6** and **7**, and obviously correlate with the stereochemistry of the epoxide function.

A qualitative explanation for the observed differences in chemical shifts for C-17, C-21 and C-23 in the epimeric epoxides could be attributed to the predominance of a given conformation of the side chain in each compound. It is reasonable to expect, as previously suggested,[13,14] that the preferred conformation around C-20/C-22 of the *R,R*-epoxide corresponds to the Newman projection **I**. In this conformation the oxirane ring has a parallel 1,3-interaction with the hydrogens of C-21 and C-17, consequently the signals of both carbons should be shielded by the well-known oxirane effect.[9] In the case of the *S,S*-epimer the more favorable conformation could be better represented by the Newman projection **II**, closely similar to that adopted by the side chain of **3**. In this conformation the oxirane ring still has a parallel 1,3-interaction with the C-21 hydrogens but not with that of C-17, therefore, only C-21 should suffer the shielding effect, in agreement with the observed chemical shift values for **5** compared with those of **3**. In conclusion the oxirane ring effect produces upfield shifts of the expected magnitudes (*ca* 5 ppm) at C-21 in both series, whereas to a lesser extent (*ca* 2.5 ppm) only at C-17 for the *R,R*-epimers. This apparent difference in magnitudes of shielding effects could be attributed to the additional interaction between C-17 and C-23 present in conformation **II**, that also explains the upfield shift observed at the latter carbon of this series compared with the same site (C-23) of the *R,R* series.

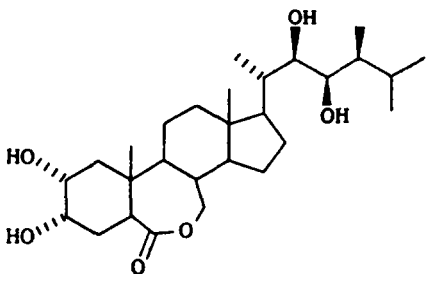
In order to extend these observations we have also studied the epimeric epoxides of 3α,5α-cycloergost-7,22-dien-6-one (**9** and **10**).[15] The effects on C-17, C-21, C-22 and C-23 are in agreement with those observed for the stigmast-22-en epoxides and indicate that they are, as expected, independent of the stereochemistry at C-24 and that could be of diagnostic value in distinguishing epoxides of different stereochemistry. It is worthwhile mentioning that recently the interesting compound **12**, a possible intermediate in the biogenesis of 23-oxo-steroids, has been isolated from a marine sponge.[16] Unfortunately, the $^{13}$C-NMR data are not available since, if our suggestions are correct, the stereochemistry of the natural product could be easily determined.

The carbon shifts of **4–11** assigned by comparison with related compounds previously reported[17,18] and by analysis of the generated CH/CH$_3$ and CH$_2$/q subspectra by spin–echo sequences utilizing the proton-flip method (APT)[19] are listed in Table 1.

## EXPERIMENTAL

The $^1$H-NMR were recorded at 80.13 MHz and the $^{13}$C-NMR spectra at 20.15 MHz in the Fourier transform mode and in $CDCl_3$ solns. Chemical shifts are expressed on the TMS scale according to: TMS = $\delta$ $CDCl_3$ + 76.9 ppm. TLC was done on silica gel GF 254 and column chromatography on silica gel H.

Compounds **4** and **5** have been prepared by direct epoxidation of i-stigmasteryl methyl ether[20] with *m*-CPBA in $CH_2Cl_2$ soln overnight, followed by careful column

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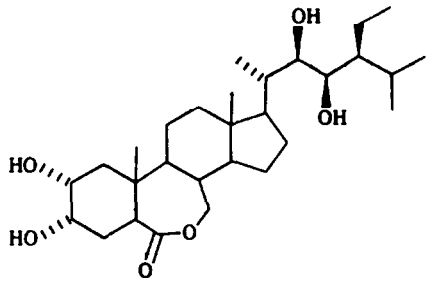


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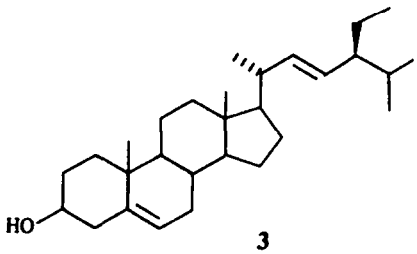


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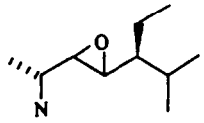


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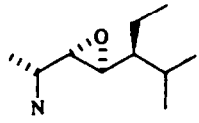


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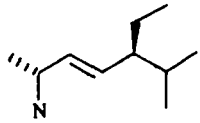


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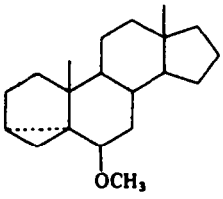


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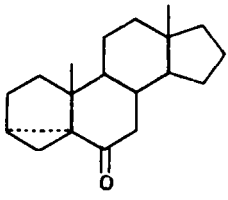


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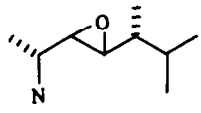


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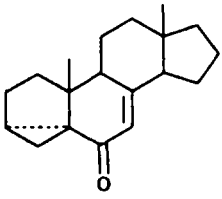


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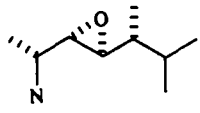


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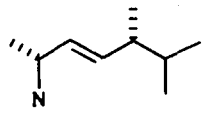


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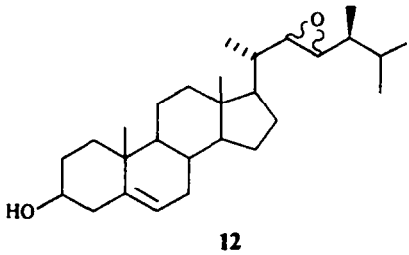


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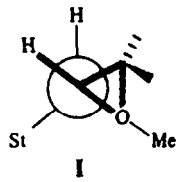


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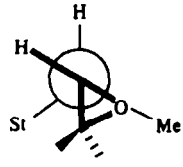


II

Table 1. Carbon shifts of compounds studied

| Carbon No. | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|
| 1 | 33.2 | 33.3 | 33.3 | 33.4 | 33.4 | 33.6 | 33.5 | 33.5 |
| 2 | 24.2 | 24.3 | 24.1 | 24.1 | 24.0 | 26.6 | 26.2 | 26.5 |
| 3 | 21.3 | 21.4 | 35.1 | 35.0 | 35.1 | 34.6 | 34.5 | 34.5 |
| 4 | 12.9 | 12.9 | 11.5 | 11.5 | 11.5 | 13.0 | 12.8 | 12.8 |
| 5 | 35.0 | 35.3 | 46.1 | 46.2 | 46.2 | 44.8 | 44.8 | 44.8 |
| 6 | 82.2 | 82.2 | 209.1 | 209.1 | 209.5 | 196.6 | 196.6 | 196.6 |
| 7 | 34.9 | 34.9 | 46.5 | 46.5 | 46.1 | 123.5 | 123.5 | 123.3 |
| 8 | 30.3 | 30.4 | 34.6 | 34.6 | 34.7 | 163.3 | 163.4 | 163.8 |
| 9 | 48.1 | 48.0 | 46.0 | 46.0 | 46.1 | 41.8 | 41.8 | 41.7 |
| 10 | 42.8 | 43.0 | 42.1 | 42.6 | 42.5 | 44.5 | 44.5 | 44.0 |
| 11 | 22.5 | 22.6 | 22.7 | 22.7 | 22.8 | 22.9 | 22.7 | 22.5 |
| 12 | 39.9 | 40.1 | 39.4 | 39.5 | 39.6 | 38.8 | 38.8 | 38.7 |
| 13 | 43.2 | 43.7 | 44.6 | 44.6 | 44.7 | 43.5 | 43.5 | 43.4 |
| 14 | 56.3 | 56.4 | 56.5 | 56.5 | 57.0 | 55.4 | 55.9 | 55.8 |
| 15 | 24.7 | 24.8 | 25.7 | 25.7 | 25.8 | 22.9 | 22.9 | 22.9 |
| 16 | 27.8 | 27.1 | 27.7 | 26.8 | 28.7 | 27.2 | 26.6 | 27.6 |
| 17 | 53.5 | 56.1 | 53.3 | 55.9 | 55.8 | 53.5 | 55.4 | 55.8 |
| 18 | 12.0[a] | 12.2 | 11.9[a] | 12.0[a] | 12.1 | 12.4 | 12.4 | 12.4 |
| 19 | 19.4[b] | 19.3[a] | 19.5[b] | 19.3 | 20.9[a] | 19.2[a] | 19.1[a] | 19.1[a] |
| 20 | 38.4 | 38.7 | 38.4 | 38.6 | 40.3 | 39.4 | 38.7 | 40.8 |
| 21 | 15.9 | 16.1 | 15.9 | 16.1 | 21.1 | 16.8 | 16.1 | 20.8 |
| 22 | 61.7[c] | 62.9 | 61.8 | 62.7 | 137.8 | 62.4[b] | 63.6 | 134.8 |
| 23 | 61.8[c] | 58.3 | 61.8 | 58.4 | 129.5 | 63.5[b] | 60.1 | 132.1 |
| 24 | 47.9 | 48.6 | 48.1 | 48.6 | 51.1 | 42.2 | 42.0 | 42.5 |
| 25 | 28.9 | 29.2 | 29.0 | 29.2 | 31.7 | 30.8 | 30.9 | 32.8 |
| 26 | 19.0[b] | 19.1[a] | 19.5[b] | 19.3 | 18.9[a] | 18.4[a] | 19.3[a] | 19.4[a] |
| 27 | 19.9[b] | 19.3[a] | 20.0[b] | 19.3 | 19.5[a] | 20.0[a] | 20.8[a] | 19.7[a] |
| 28 | 20.7 | 20.9 | 20.7 | 20.8 | 25.3 | 12.4 | 13.4 | 17.3 |
| 29 | 12.2[a] | 12.2 | 12.3[a] | 12.2[a] | 12.1 | | | |
| $OCH_3$ | 55.9 | 56.0 | | | | | | |

[a–c] The assignments for these signals within a vertical column may be reversed.

chromatography. The less polar epoxide **4** crystallized from acetone, m.p. 89–90°. $^1$H-NMR $\delta$: 2.49 (m, 1H), 2.65–2.79 (m, 2H), 3.32 (s, 3H). MS: 442 ($M^+$). (Found: C, 81.55; H, 11.08. Calc for $C_{30}H_{50}O_2$: C, 81.38; H, 11.38%.) The more polar epoxide **5** is an oil. $^1$H-NMR: 2.40–2.60 (m, 2H), 2.76 (br, 1H), 3.32 (s, 3H). MS (high resolution): found 442.3743, calc for $C_{30}H_{50}O_2$ 442.3813.

By epoxidation of **8**[7] with *m*-CPBA in $CH_2Cl_2$ overnight followed by careful column chromatography epoxides **6** and **7**, and a small amount of (22*R*,23*R*)-**13** were isolated. Epoxide **6** is an oil. $^1$H-NMR $\delta$: 2.50 (m, 1H), 2.74 (dd, 1H). MS (high resolution): found 426.3515, calc for $C_{29}H_{46}O_2$ 426.3498. Epoxide **7** is an oil. $^1$H-NMR $\delta$: 2.40–2.60 (m, 2H). MS (high resolution): found 426.3527, calc for $C_{29}H_{46}O_2$ 426.3498. Lactone **13** is an oil. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1750. $^1$H-NMR $\delta$: 2.50 (m, 2H), 2.73 (dd, 1H). $^{13}$C-NMR: 53.5 (C-17), 61.7 (C-22), 61.7 (C-23). MS (high resolution): found 442.3526, calc for $C_{29}H_{46}O_3$ 442.3449.

Compounds **9–11** were prepared from ergosterol according to reported procedures. Epoxide **9**, m.p. 156–159° (lit.[21] m.p. 158–160°). Epoxide **10**, m.p. 176–179° (lit.[21] m.p. 179–182°).

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