Phytochemistry, 1969, Vol. 8, pp. 463 to 467. Pergamon Press. Printed in England

# ISOMERS OF 24-ETHYLIDENECHOLESTEROL: GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC CHARACTERIZATION

B. A. KNIGHTS and C. J. W. BROOKS

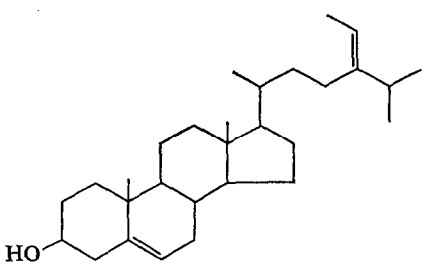
Departments of Botany and Chemistry, University of Glasgow, Glasgow, W.2

(*Received* 9 *August* 1968)

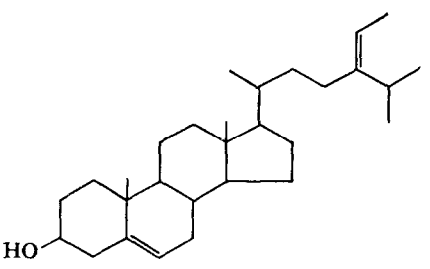
**Abstract**—The C-28 isomers of 24-ethylidenecholesterol, $\Delta^5$-avenasterol and fucosterol, are characterized by gas chromatography and mass spectrometry. Similar results are quoted for one isomer of 24-ethylidenelophenol (citrostadienol) and 24-ethylidene-$\Delta^7$-cholesten-3$\beta$-ol ($\Delta^7$-avenasterol).

## INTRODUCTION

THE BIOSYNTHESIS of plant sterols, involving introduction of an ethyl group at $C_{24}$, has been shown to proceed via a $C_{24}$-ethylidene sterol intermediate in the phytoflagellate *Ochromonas malhamensis*,[1] whereas in the slime mould *Dictyostelium discoideum* it has been shown that such an intermediate is not directly involved in the formation of a C-24 ethyl group.[2] Two isomers at C-28 are possible for 24-ethylidenecholesterol and both are known, naturally occurring sterols. Thus fucosterol (I) was first isolated from the *Phaeophyceae*[3] and $\Delta^5$-avenasterol (II), the second most abundant sterol in seed of *Avena sativa*,[4, 5] was synthesized from 24-ketocholesterol by a Wittig reaction.[6] The stereochemistry of the biosynthesis of these two compounds has been discussed in detail,[7] and further interest arises from their physiological action in inducing sexual reproduction of the Oomycete fungus *Phytophthora cactorum*.[8] Both sterols are effective in facilitating formation of antheridia and oogonia but only $\Delta^5$-avenasterol is effective in promoting formation of mature oospores.




Fucosterol (I)




$\Delta^5$-Avenasterol (II)

[1] A. R. H. SMITH, L. J. GOAD, T. W. GOODWIN and E. LEDERER, *Biochem. J.* **104**, 56C (1967).
[2] M. LENFANT, E. ZISSMANN and E. LEDERER, *Tetrahedron Letters* 1049 (1967).
[3] I. M. HEILBRON, R. F. PHIPERS and H. R. WRIGHT, *J. Chem. Soc.* 1572 (1934).
[4] D. R. IDLER, S. W. NICKSIC, D. R. JOHNSON, V. W. MELOCHE, H. A. SCHUETTE and C. A. BAUMANN, *J. Am. Chem. Soc.* **75**, 1712 (1953).
[5] B. A. KNIGHTS, *Phytochem.* **4**, 857 (1965).
[6] J. P. DUSZA, *J. Org. Chem.* **25**, 93 (1960).
[7] G. F. GIBBONS, L. J. GOAD and T. W. GOODWIN, *Phytochem.* **7**, 983 (1968).
[8] C. G. ELLIOTT, M. R. HENDRIE and B. A. KNIGHTS, *J. Gen. Microbiol.* **42**, 425 (1966).

Thus it is desirable to be able to recognize and characterize these two compounds and related 24-ethylidene sterols (e.g. citrostadienol and $\Delta^7$-avenasterol). For compounds of reasonable purity, NMR spectra serve to distinguish the two C-28 isomers of 24-ethylidenecholesterol, satisfactory spectra for the two compounds having been recorded previously.[7,9] Double irradiation experiments have confirmed that the heptet for the C-25 proton occurs at 7·2 $\tau$ for $\Delta^5$-avenasterol and at 7·8 $\tau$ for fucosterol,[10] and these results have been taken to show[10,11] that the stereochemistry at C-28 is the opposite of that previously described.[5–7] The mass spectrum of $\Delta^5$-avenasterol, isolated from the green algae *Enteromorpha intestinalis* and *Ulva lactuca*,[7] and of the derived trimethylsilyl ether[12] have been recorded and show ions characteristic of the side-chain double bond. The fragmentations involved have been the subject of detailed analysis.[13,14]

It is the purpose of this paper to record and compare the GLC and mass spectrometric data to enable these and related compounds to be identified more readily when they occur in the complex mixtures of sterols often found in higher plants.

## RESULTS AND DISCUSSION

Table 1 lists the retention indices found on a number of columns for the two isomers of 24-ethylidenecholesterol. The corresponding data obtained on OV-17 for 24-ethylidene-$\Delta^7$-cholesten-3$\beta$-ol ($\Delta^7$-avenasterol) citrostadienol (4$\alpha$-methyl-$\Delta^7$-avenasterol, 24-ethylidenelophenol) and several other sterols are listed in Table 2. It may be seen from Table 1 that the $\Delta^5$-avenasterol isomer is consistently the more polar compound, and the most satisfactory separation is obtained on the thermostable poly alkyl/aryl siloxane (50% phenyl groups) OV-17 (see Fig. 1). The $\Delta I_r^{OV-17}$ value (24-methylene → 24-ethylidene)[15] for fucosterol is

TABLE 1. RETENTION INDICES OF 24-ETHYLIDENECHOLESTEROL ISOMERS

| Stationary phase | Length of column (ft) | Temperature (°C) | Fucosterol TMSi ether | 28-isoFucosterol TMSi ether |
|---|---|---|---|---|
| 1% SE-30 | 9 | 248 | 3300 | 3310 |
| 1% DC-560 | 4 | 225 | 3265 | 3280 |
| 1% DC-710 | 5 | 225 | 3370 | 3380 |
| 5% FS-1265 | 9 | 228 | 3485 | 3505 |
| 0·1% OV-17 | 9* | 225 | 3400 | 3420 |
| 3% OV-17 | 9 | 255 | 3460 | 3475 |
| 1% HiEFF-8B | 4 | 225 | 3560 | 3580 |
| 1% HiEFF-8B/2% PVP | 9 | 228 | 3420 | 3440 |
| 1% HiEFF-8B/2% Polysulphone | 5 | 244 | 3460 | 3485 |
| 1% EGSP-Z | 4 | 210 | 3500 | 3520 |

* Glass beads.

[9] W. R. NES, M. CASTLE, J. L. MCCLANAHAN and J. M. SETTINE, *Steroids* **8**, 655 (1966).
[10] R. B. BATES and J. W. ROWE, unpublished results.
[11] D. J. FROST and J. P. WARD, *Tetrahedron Letters* 3779 (1968).
[12] B. A. KNIGHTS, *J. Gas Chromatog.* **5**, 273 (1967).
[13] S. G. WYLLIE and C. DJERASSI, *J. Org. Chem.* **33**, 305 (1968).
[14] C. J. W. BROOKS, E. C. HORNING and J. S. YOUNG, *Lipids*, in press.
[15] B. A. KNIGHTS, *J. Gas Chromatog.* **4**, 329 (1966).

95 RI units and for $\Delta^5$-avenasterol 110 RI units. The corresponding value observed for citrostadienol was 110 RI units, consistent with its having the same stereochemistry as $\Delta^5$-avenasterol. Similar data were found for the samples of 4α-methyl-$\Delta^7$-avenasterol obtained from *Solanum tuberosum*, birch and elm.[16] Double irradiation NMR spectra have confirmed all these assignments of relative stereochemistry by demonstrating the heptet for the C-25 hydrogen at 7·2 $\tau$.[10]

TABLE 2. RETENTION INDICES OF SOME STEROL TMSi ETHERS ON 3% OV-17 (255°)

| | |
|---|---|
| 24-Methylenecholesterol | 3365 |
| β-Sitosterol | 3440 |
| 24-Ethylidene-$\Delta^7$-cholesten-3β-ol ($\Delta^7$-avenasterol) | 3540 |
| 24-Methylene-4α-methyl-$\Delta^7$-cholesten-3β-ol | 3505 |
| 24-Ethylidene-4α-methyl-$\Delta^7$-cholesten-3β-ol (citrostadienol) | 3615 |

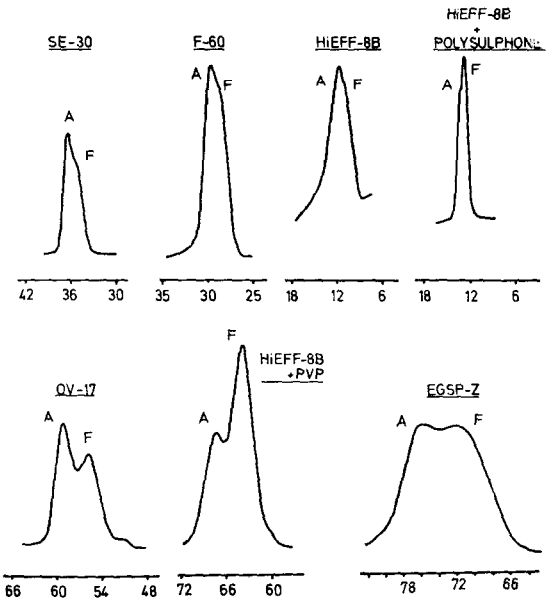



FIG. 1. GLC SEPARATIONS OF FUCOSTEROL (F) AND $\Delta^5$-AVENASTEROL (A) AS THE TRIMETHYLSILYL ETHERS.

Figure 2 shows the mass spectra of the derived acetates of fucosterol and $\Delta^5$-avenasterol. No molecular ion was detected in either mass spectrum, and previous experience has shown that loss of acetic acid to produce a parent ion corresponding to M-60 is usual for acetate derivatives of 3β-hydroxy-$\Delta^5$-steroids. The relative intensities of the ions at *m/e* 55, 296 and 394 are consistently different for the two compounds. Under the conditions used in this work 296 was always the base peak of spectra from $\Delta^5$-avenasterol acetate and 55 the base peak of

[16] Donation of these samples by K. SCHREIBER, B. O. LINDGREN and J. W. ROWE respectively is gratefully acknowledged.

spectra corresponding to fucosterol acetate. The relatively more facile loss of 98 mass units ($C_7H_{14}$)[7, 12, 13, 17, 18] from $\Delta^5$-avenasterol is possibly a reflection of the greater ease of transfer of the C-20 hydrogen atom to $C_{28}$, via a McLafferty type rearrangement, when the methyl and isopropyl groups have the *cis* configuration.[19] Mass spectra of trimethylsilyl ether derivatives exhibit less clear-cut differences than do the acetates. The mass spectrum for $\Delta^5$-avenasterol trimethylsilyl ether has been recorded in the range *m/e* 200 $-M^+$.[12] Since only one isomer of each of 24-ethylidene-$\Delta^7$-cholesten-3$\beta$-ol and the corresponding 4$\alpha$-methyl sterol have been described (each having the same relative configuration at C-28) it is not possible to say whether any consistent differences are to be found in mass spectra of the geometrical isomers. As with other $\Delta^7$-sterols[12] there is a marked orientation of the fragmentation pattern towards

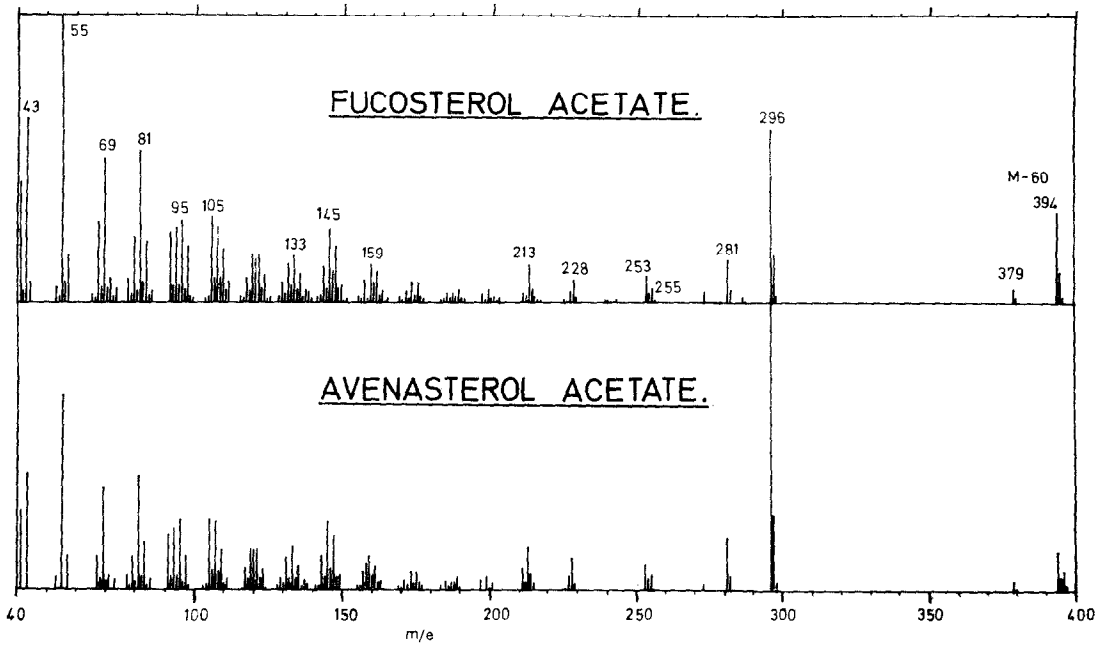



FIG. 2. MASS SPECTRA OF FUCOSTEROL ACETATE AND $\Delta^5$-AVENASTEROL ACETATE.

cleavage of the C-17–C-20 bond and, similarly with other diunsaturated sterols,[12–14] this cleavage is largely accompanied by loss of two nuclear hydrogen atoms, a process already observed as characteristic of desmosterol and related sterols with unsaturated side-chains. Thus the two largest ions recorded in the high mass range of spectra of both $\Delta^7$-compounds are derived respectively from loss of 141 and 98 mass units from the molecular ion.[12]

It may be concluded that the two isomers of 24-ethylidenecholesterol may be recognized in mixtures by careful gas chromatography on stationary phases such as Hi-EFF-8B, HiEFF-8B/PVP and especially OV-17 and that mass spectrometry of the acetates further facilitates the identification.

[17] J. BERGMAN, B. O. LINDGREN and C. M. SVAHN, *Acta. Chem. Scand.* **19**, 1661 (1965).
[18] P. BENVENISTE, L. HIRTH and G. OURISSON, *Phytochem.* **5**, 31 (1966).
[19] A. MCCORMICK, personal communication.

## EXPERIMENTAL

Fucosterol was isolated from *Fucus spiralis* and $\Delta^5$-avenasterol from seed of *Avena sativa* L. as described previously.[5]

Acetate derivatives were prepared by a standard technique.[20] GLC column packings were prepared by standard techniques,[21] phases being purchased from Applied Science Laboratories. The packing 3% OV-17 coated on Gas-Chrom Q was purchased as a pretested packing from Applied Science Laboratories. Two types of instrument were used: Pye Argon chromatographs with 4-ft columns adapted for use with a self-sealing septum injection system and equipped with preheaters, and a Pye 104 chromatograph with 5-ft and 9-ft columns. Operating conditions are mentioned in Table 1.

Mass spectra were obtained on an LKB 9000 mass spectrometer using a column packed with SE-30 stationary phase at 250°. Molecular separators were operated at 250° and the ion source at 275° with a scan voltage of 70 eV.

*Acknowledgements*—An LKB 9000 combined gas chromatograph mass spectrometer was purchased under S.R.C. grant number B/SR/2398 to Drs. C. J. W. Brooks and G. Eglinton. The technical assistance of Miss H. Humphrys and Miss J. Malcolm is gratefully acknowledged.

[20] B. A. KNIGHTS, *Memoirs of the Society for Endocrinology No.* 16 (edited by J. K. GRANT) p. 211 (1967).
[21] E. C. HORNING, W. J. A. VANDEN HEUVEL and B. G. CREECH, *Methods of Biochemical Analysis*, XI (edited by D. GLICK), p. 69, Wiley, New York (1963).