Phytochemistry, 1971, Vol. 10, pp 3135 to 3137. Pergamon Press. Printed in England.

SHORT COMMUNICATION

# STEROLS IN GRASS SEEDS

BERNARD N. BOWDEN and PAUL M. WILLIAMS

Chelsea College of Science and Technology, Hortensia Road, London SW 10

(*Received* 1 *April* 1971)

**Abstract**—The sterol contents of the seeds of twelve representative species of grasses were determined by gas chromatography. All contained β-sitosterol, campesterol and stigmasterol, whereas cholesterol, $\Delta^5$- and $\Delta^7$-avenasterols were detected in 10, 10 and 2 species respectively.

## INTRODUCTION

IT WAS thought for many years that the principal sterols in the Gramineae were β-sitosterol, stigmasterol and campesterol.[1–4] In 1953[5] two new sterols were isolated from oat seeds by means of column chromatography. These were named $\Delta^5$- and $\Delta^7$-avenasterols, since the Liebermann–Burchard reaction indicated a double bond in the nucleus at carbons five and seven respectively. Both avenasterols were shown to contain twenty nine carbon atoms and a second double bond which was thought to be situated at carbon twelve. Both avenasterols were present in significant quantities, the $\Delta^5$- sterol making up 32% and the $\Delta^7$-sterol 11% of the total sterol content.

Knights[6] later found that it was possible to separate the two avenasterols by GLC on a polar column and confirmed their presence in oat seeds. However, using chemical and mass-spectrographic evidence, Knights concluded that the second double bond was located in the side chain between carbons twenty four and twenty eight so that the structures of the avenasterols became $\Delta^{5,24(28)}$- and $\Delta^{7,24(28)}$-stigmastadienol.

Cholesterol, formerly thought to be an exclusively animal sterol, has now been shown to be present in a variety of plants.[2,7,8]

In the light of these developments, an investigation was made of the sterols in the seeds of twelve species representing six major tribes of the Gramineae.

## RESULTS AND DISCUSSION

A total lipid isolate was prepared by extracting 4 g of seed with a mixture of chloroform and methanol.[9,10] The sterols were then separated from other lipids on silica gel plates developed with a petrol ether–diethyl ether–acetic acid mixture.[11] After spraying with Rhodamine

[1] W. BERGMAN, *Ann. Rev. Plant Physiol.* **4**, 383 (1953).
[2] E. HEFTMANN, *Ann. Rev. Plant Physiol.* **14**, 225 (1963).
[3] A. HEUSNER, in *Encyclopedia of Plant Physiology* (edited by W. RUHLAND), Vol. X, p. 132, Springer–Verlag (1958).
[4] R. HEGNAUER, *Chemotaxonomie der Pflanzen*, Birkhauser, Basle (1962).
[5] D. R. IDLER, S. W. NICKS, D. R. JOHNSON, V. W. MELOCHE, H. A. SCHUETTE and C. A. BAUMAN, *J. Am. Chem. Soc.* **75**, 1712 (1953).
[6] B. A. KNIGHTS, *Phytochem.* **4**, 857 (1965).
[7] K. TSUDA, S. AGAKI, Y. KISHIDA, R. HAYATSU and K. SAKAI, *Chem. Pharm. Bull. Tokyo* **6**, 724 (1958).
[8] D. F. JOHNSON, R. D. BENNET and E. HEFTMANN, *Science* **140**, 198 (1963).
[9] J. FOLCH, M. LEES and G. H. SLOANE STANLEY, *J. Biol. Chem.* **226**, 497 (1957).
[10] G. ROUSER, G. KRITCHEVSKY, D. HELLER and E. LIEBER, *J. Am. Oil. Chem. Soc.* **40**, 425 (1963).
[11] D. C. MALINS and H. K. MANGOLD, *J. Am. Oil. Chem. Soc.* **37**, 576 (1960).

6G[12] and viewing by UV light the sterol band was able to be scraped off the plate, the component sterols eluted and separated by gas chromatography.

It was necessary to use two columns to obtain an adequate separation. The E301 column, which is non-polar, separated cholesterol, campesterol, stigmasterol and β-sitosterol, but two other sterols, X and Y, were not resolved. The second column, Carbowax 20M–TPA, is polar and resolved X and Y but did not separate campesterol from stigmasterol.

Identifications were made using the relative retention times of the sterols on both columns and of the trimethylsilyl derivatives on the E301 column. Estimates of the relative quantities of the sterols were made from measurements of peak areas multiplied by the weight of the sterol standard divided by the peak area of the standard.

All twelve grass seeds contained the three commonest plant sterols; β-sitosterols, stigmasterol and campesterol (Table 1). β-Sitosterol made up about half the total sterols by weight while stigmasterol and campesterol each made up about one fifth of the total.

Small quantities of cholesterol were detected in ten of the twelve seeds; most containing less than 2·5% but over 6% cholesterol was found in *Panicum maximum*. The two other sterols, X and Y, were found in the seeds of several species.

When Knights[6] separated the acetates of oat seed sterols on an HiEFF8B- PVP polar column, he showed that in addition to the cholesterol, brassicasterol, campesterol, stigmasterol and β-sitosterol peaks there were also two other sterols present which he identified as $\Delta^5$- and $\Delta^7$-avenasterols.

As authentic samples of these two avenasterols were not available, the sterols were extracted from oat seeds and separated on the Carbowax 20M–TPA column. The major oat sterols, including the two avenasterols, could be readily identified from their relative retention times and peak areas on the chromatogram. As they were extracted and separated by exactly the same procedures as the X and Y sterols, direct comparisons could be made.

TABLE 1. THE STEROL CONTENTS OF TWELVE GRASS SEEDS

| | | | Per cent by weight of total sterols | | | | | |
|---|---|---|---|---|---|---|---|---|
| Subfamily | Tribe | Species | Cholesterol | Campesterol | Stigmasterol | β-Sitosterol | (X) $\Delta^5$-Avenasterol | (Y) $\Delta^7$-Avenesterol |
| Festucoideae | Agrosteae | *Agrostis tenuis* | 1 29 | 18 26 | 14 74 | 57 98 | 7 16 | N.D |
| | Phalarideae | *Phalaris tuberosa* | N D. | 20 25 | 10 62 | 58 44 | 10 69 | N D |
| Chloridoideae | Eragrosteae | *Eragrostis curvula* | 0 95 | 15 47 | 11 77 | 44 51 | 26 82 | 0·48 |
| | Chlorideae | *Chloris gayana* | 1 09 | 14 95 | 20 14 | 58 35 | 5 47 | N D |
| Panicoideae | Paniceae | *Panicum coloratum* | 6 73 | 13 81 | 19 92 | 54 82 | 4 72 | N D. |
| | | *Setaria sphacelata* | 1 85 | 23 21 | 24 49 | 41 50 | 8 95 | N.D. |
| | | *Brachiaria ruziziensis* | 2 79 | 11 82 | 15 54 | 55 65 | 13 6 | 0 6 |
| | | *Melinis minutiflora* | 0 56 | 20 30 | 21 74 | 57 40 | N D. | N D |
| | Andropogoneae | *Sorghum vulgare* | N D | 25 88 | 20 62 | 45 94 | 7 56 | N D. |
| | | *Sorghum sudanense* | 0 17 | 20 60 | 29 87 | 46 96 | 2 40 | N D. |
| | | *Sorghum alum* | 0 56 | 19 08 | 38 53 | 38 83 | 3 00 | N D |
| | | *Andropogon gayanus* | 0 91 | 17 62 | 16 74 | 64 73 | N D | N D. |

[12] G. V. MARINETTI, *J. Lipid Research* **3**, 1 (1962).

Close agreement was found in all cases between the relative retention times and peak shapes of X with the $\Delta^5$- and Y with the $\Delta^7$-avenasterols on the oat chromatograms. It therefore seems probable because of this agreement and their similar origin in gramineous seeds that X is the $\Delta^5$ and Y the $\Delta^7$ avenasterol identified by Knights.[6]

The $\Delta^5$-avenasterol was present in all the grass seeds examined except for *Melinis minutiflora* and *Andropogon gayanus*. The quantity present varied considerably reaching 27% in *Eragrostis curvula* and 14% in *Brachiaria ruziziensis*. The $\Delta^7$-avenasterol was detected only in these same two species and made up about 0·5% of the total sterol.

As $\Delta^7$-avenasterol was found to be present in much smaller quantities than $\Delta^5$-avenasterol in all the species where both were detected and since the $\Delta^7$-avenasterol was detected only in seed containing the largest quantities of $\Delta^5$-avenasterol, it is possible that more sensitive methods might reveal $\Delta^7$-avenasterol in other species containing $\Delta^5$-avenasterol.

It therefore seems probable that cholesterol and the two avenasterols are more widely distributed in the Gramineae than was previously realised.

## EXPERIMENTAL

*Source of the seed material.* The seeds were purchased from the Kenya Seed Co., Ltd., Kitale, Kenya except for *Sorghum vulgare* DC 36 which was purchased from Gunsons Seeds South Africa (Pty.) Ltd., Johannesburg and *Andropogon gayanus* var. *bisquamulatus* which was kindly donated by Mr. R. J. Haggar, Shika Experimental Station, Northern Nigeria.

*Extraction and separation of the sterols.* 4 g dry wt. of seeds was extracted with $CHCl_3$–MeOH mixture[9, 10] and concentrated under $N_2$. May and Baker Chromolay TLC plates layered with Silica Gel G (Merck) 250 $\mu$ thick were pre-developed in $Et_2O$ and dried. The extract was then streaked on and the plates developed in petrol ether –$Et_2O$–HOAc (90: 10: 1).[11] The resulting bands were detected by spraying with 0·0012% aq. Rhodamine 6G[12] and viewing in UV light. The sterol band was then scraped off and the sterols eluted in $Et_2O$.

The sterols were finally separated on a Perkin–Elmer F11 gas chromatograph equipped with a flame ionization detector and injection port temp. of 280°. Two columns were used. E 301 (non-polar) 1 m, 0·125 in O.D., at 215° with $N_2$ carrier gas fed at 18 ml/min and Carbowax 20M–terepthalic acid (polar) as before with $N_2$ fed at 55 ml/min. Trimethylsilyl derivatives of the sterols[13] were also separated on the E 301 column.

[13] F. A. VANDENHEUVEL, G. J. HINDHERKS, J. C. NIXON and W. G. LAYNG, *J. Am. Oil. Chem. Soc.* **42**, 283 (1965).