Phytochemistry, Vol. 22, No. 3, pp. 789–790, 1983.
Printed in Great Britain.

0031–9422/83/030789–02$03.00/0

ISOLATION OF 22-DEHYDROCAMPESTEROL FROM THE SEEDS OF *BRASSICA JUNCEA*

T. MATSUMOTO,‡ N. SHIMIZU, T. SHIGEMOTO, T. ITOH, T. IIDA* and A. NISHIOKA†

College of Science and Technology, Nihon University, Kanda Surugadai, Chiyoda-ku, Tokyo 101, Japan; *College of Engineering, Nihon University, Koriyama, Fukushima-ken 963, Japan; †Oil and Fat Research Laboratories, Ajinomoto Co. Inc., Tsurumi-ku, Yokohama 230, Japan

(*Received* 26 *July* 1982)

Key Word Index—*Brassica juncea*; Cruciferae; seeds; sterol; 22-dehydrocampesterol; brassicasterol.

Abstract—22-Dehydrocampesterol [(24*S*)-24-methylcholesta-5, *E*-22-dien-3β-ol] was isolated from the seeds of oriental mustard, *Brassica juncea*, by reversed phase HPLC. This sterol has been previously detected in some marine organisms, but never found in higher plants. The configuration at C-24 was unambiguously proved by high resolution (400 MHz) ^{1}H NMR spectroscopy.

INTRODUCTION

In a previous paper [1], we proposed that the $C_{28}\Delta^{5,22}$-sterol fraction in the unsaponifiable lipid of the seeds of *Brassica* species (rape and mustard) contains 22-dehydrocampesterol [(24*S*)-24-methylcholesta-5,*E*-22-dien-3β-ol] as a minor component (*ca* 10–30%) together with brassicasterol (24*R*-epimer). The assignment was based on the ^{13}C NMR spectral analysis. We report here the isolation and the structural confirmation of 22-dehydrocampesterol in the seeds of oriental mustard, *B. juncea*.

RESULTS AND DISCUSSION

The $C_{28}\Delta^{5,22}$-steryl acetate fraction (6 mg) separated from *B. juncea* as described in a previous paper [1] was saponified. The free sterol fraction was recovered (5 mg) and subjected to reversed phase HPLC on a Partisil 5 ODS-2 (or LiChrosorb RP-18) column, eluted with methanol–water (98 : 2) as mobile phase, to give two well resolved peaks with the ratio of 32 : 68. The two compounds were collected by prep. HPLC and further purified by re-chromatography to yield a faster eluted minor component **1** (*ca* 1 mg) and the main component **2** (*ca* 2 mg).

The GC RR_ts on an OV-1 capillary column for the acetates of **1** and **2** were 1.097 and 1.104, respectively. The free sterol **1** showed a mp 160.5–161.5° which differs from the reported mps for the C-24 epimer of brassicasterol, i.e. pincsterol (136–137°) [2] from pearl oyster (*Pinctada martensi*), crinosterol (137–138°) [3] from crinoid (*Comatula*) and a sterol (147–148°) [4] from a diatom (*Phaeodactylum tricornum*), though the acetate of **1** (156–157°) was nearly identical to the mp given for a steryl acetate (157–158°) [4] from the diatom. The mps for the free sterol **2** (151–152°) and its acetate (157–158°) were in accord with the reported values [5] for brassicasterol (150–151°) and its acetate (157.5–158.5°), respectively. The mass spectra of both **1** and **2**, as well as of their acetates, were very similar to each other.

Inspection of 400 MHz ^{1}H NMR spectra of **1** and **2** (Table 1), however, revealed a significant difference serving to characterize the structures as well as the configurations at C-24. Whereas the C-21 methyl signal for **2** (δ 1.011) appears at lower field by δ 0.01 than that for **1** (δ 1.001), the resonance positions of the remaining methyls are virtually the same for both the compounds. The chemical shift values and coupling constants of the methyls for **1** and **2** were in good agreement with the published data for 22-dehydrocampesterol and brassicasterol [6, 8–10], respectively. The minor component **1** in the *B. juncea* $C_{28}\Delta^{5,22}$-sterol fraction was thus confirmed as 22-dehydrocampesterol.

This appears to be the first report of the isolation of 22-dehydrocampesterol from a higher plant. This sterol has so far been identified in diatoms and some marine invertebrates [2–4, 6, 7]. The co-occurrence of C-24 epimeric 24-methyl-$\Delta^{5,22}$-sterols in *Brassica* seeds may help to further clarify the mode of biosynthesis of the phytosterol side chain in higher plants.

EXPERIMENTAL

Mps are uncorr. MS (direct inlet) were determined at 70 eV. HPLC was carried out on a Partisil 5 ODS-2 (Whatman, 8 mm i.d. × 25 cm; packed by Erma Optical Works, Tokyo, Japan) or LiChrosorb RP-18 (Merck, 4.6 mm i.d. × 20 cm; laboratory-packed), using a Kyowa Seimitsu double plunger pump and a Shimadzu SPD-2AS UV detector monitoring at 210 nm (flow rate, 2 ml/min; MeOH–H_2O, 98 : 2, as mobile phase). For prep. purposes, 0.5 ml aliquots of sample soln (5 mg/10 ml MeOH) were each injected to the ODS-2 column, and the two fractions

‡Author to whom correspondence should be addressed.

Table 1. 400 MHz ^{1}H NMR methyl group chemical shifts of the C-24 epimeric C_{28} $\Delta^{5,22}$-sterols isolated from *Brassica juncea*

	C-18	C-19	C-21	C-26	C-27	C-28
22-Dehydrocampesterol (1)	0.692 (*s*)	1.009 (*s*)	1.001 (*d*, J = 6.35)	0.834 (*d*, J = 7.33)	0.817 (*d*, J = 6.83)	0.909 (*d*, J = 6.84)
Brassicasterol (2)	0.692 (*s*)	1.009 (*s*)	1.011 (*d*, J = 6.84)	0.833 (*d*, J = 6.35)	0.817 (*d*, J = 6.35)	0.909 (*d*, J = 6.84)

All chemical shifts given in δ-values from TMS; coupling constants in Hz.

were collected each time. GC was carried out on an OV-1 WCOT Si capillary column (0.25 mm i.d. × 50 m, 280°) and *RR*,s are given relative to cholesteryl acetate (1.000). ^{1}H NMR spectra were recorded at 400 MHz with a JEOL JNM FX-400 spectrometer, using $CDCl_3$ as solvent and TMS as an int. standard.

The $C_{28}\Delta^{5,22}$-steryl acetate mixture was obtained from the unsaponifiable matters of *B. juncea* seeds. Prep TLC, gave the sterols which were acetylated and further purified by $AgNO_3$–Si gel TLC as described previously [1].

22-Dehydrocampesterol (1). MS *m/z* (rel. int.): 398 $[M]^+$ (100), 383 (13), 380 (18), 365 (18), 355 (9), 337 (22), 300 (49), 271 (56), 255 (96), 213 (37). 1-acetate: 380 $[M - HOAc]^+$ (100), 365 (9), 337 (10), 282 (9), 255 (62), 228 (11), 213 (16).

Brassicasterol (2). MS *m/z* (rel. int): 398 $[M]^+$ (100), 383 (16), 380 (19), 365 (18), 355 (11), 337 (28), 300 (56), 271 (65), 255 (94), 213 (34). 2-acetate: 380 $[M - HOAc]^+$ (100), 365 (9), 337 (10), 282 (9), 255 (61), 228 (11), 213 (15).

Acknowledgements—We thank K. Matsumoto and H. Yazima for technical assistance, Dr. Y. Fujimoto for running the 400 MHz ^{1}H NMR spectra.

REFERENCES

1. Matsumoto, T., Shimizu, N., Itoh, T., Iida, T. and Nishioka, A. *J. Am. Oil Chem. Soc.* (in press).
2. Ashikaga, C. (1957) *Nippon Nogei Kagaku Kaishi* **31**, 115.
3. Bolker, H. I. (1967) *Nature (London)* **213**, 905.
4. Rubinstein, I. and Goad, L. J. (1974) *Phytochemistry* **13**, 485.
5. Kircher, H. W. and Rosenstein, F. U. (1973) *Lipids* **8**, 453.
6. Teshima, S., Patterson, G. W. and Dutky, S. R. (1980) *Lipids* **15**, 1004.
7. Morris, R. J. and Culkin, F. (1977) *Oceanogr. Mar. Biol. Annu. Rev.* **15**, 73.
8. Khalil, M. W., Idler, D. R. and Patterson, G. W. (1980) *Lipids* **15**, 69.
9. Chiu, P.-L. and Patterson, G. W. (1981) *Lipids* **16**, 203.
10. Rubinstein, I., Goad, L. J., Clague, A. D. H. and Mulheirn, L. J. (1976) *Phytochemistry* **15**, 195.

Phytochemistry, Vol. 22, No. 3, pp. 790–791, 1983.
Printed in Great Britain.

0031-9422/83/030790-02$03.00/0

A XYLOSYLGLUCOSIDE OF XANTHOXYLIN FROM *SAPIUM SEBIFERUM* ROOT BARK

ISAO KOUNO, TOSHIHIRO SAISHOJI, MIDORI SUGIYAMA and NOBUSUKE KAWANO*

Faculty of Pharmaceutical Sciences, Nagasaki University, 1–14 Bunkyo-machi, Nagasaki 852, Japan

(*Received* 11 *August* 1982)

Key Word Index—*Sapium sebiferum*; Euphorbiaceae; root bark; moretenone; moretenol; xanthoxylin; sitosterol β-D-glucoside; 2-acetyl-3,5-dimethoxyphenyl-*O*-β-D-xylopyranosyl-(1–6)-β-D-glucopyranoside; ^{1}H NMR; ^{13}C NMR.

Abstract—Besides four known compounds, a new xylosylglucoside of xanthoxylin was isolated from the root bark of Chinese tallow tree, *Sapium sebiferum* and identified as 2-acetyl-3,5-dimethoxyphenyl-*O*-β-D-xylopyranosyl-(1–6)-β-D-glucopyranoside.

INTRODUCTION

Chinese tallow tree, *Sapium sebiferum* Roxb. is common in Nagasaki city as a roadside tree. Its root bark has been used [1] in China as a purgative and diuretic. Recently, it was reported [1] to be effective against *Schistosoma japonicum*. However, only xanthoxylin (2-hydroxy-4,6-dimethoxyacetophenone) has been isolated from the root bark [2] although some other constituents, such as

*Author to whom correspondence should be addressed.