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CO-OCCURRENCE OF C-24 EPIMERIC 24-METHYL-$\Delta^{5,22}$-STEROLS IN THE SEEDS OF SOME *BRASSICA* AND *RAPHANUS* SPECIES OF CRUCIFERAE

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Key Word Index—*Brassica campestris*; *B. hirta*; *B. juncea*; *B. oleracea*; *B. napus*; *Raphanus sativus*; Cruciferae; seeds; sterol; brassicasterol; 22-dehydrocampesterol; reverse-phase HPLC.

Abstract—Reverse-phase HPLC has shown that the 24-methyl-$\Delta^{5,22}$-sterol fractions isolated from 27 seed and seed oil samples of *Brassica* and *Raphanus* species (Cruciferae) consist of *ca* 10–40% of the 24α-epimer, 22-dehydrocampesterol, in addition to the 24β-epimer, brassicasterol.

The seeds of some species of Cruciferae, e.g. *Brassica*, *Iberis* and *Raphanus*, can be characterized by the presence of appreciably large amounts (8–23%) of 24-methyl-$\Delta^{5,22}$-sterol in the 4-demethylsterol fraction of the unsaponifiable lipids [1]. After a 24β-methyl configuration was established, by chemical correlation with ergosterol (24β-methylcholesta-5,7,*trans*-22-trien-3β-ol), for the 24-methyl-$\Delta^{5,22}$-sterol (brassicasterol, **1**) isolated from the seeds of *B. rapa* [2], the 24-methyl-$\Delta^{5,22}$-sterol detected in the seeds of many Cruciferae plants was, to the best of the authors' knowledge, tentatively named 'brassicasterol' without proving the stereochemistry at C-24. Recently, we have examined by ^{13}C NMR spectroscopy the 24-methyl-$\Delta^{5,22}$-sterol fractions isolated from eight seed samples of *Brassica* sp. and demonstrated that all of the fractions were mixtures, epimeric at C-24, i.e. brassicasterol (24β-methylcholesta-5,*trans*-22-dien-3β-ol, **1**) and 22-dehydrocampesterol (24α-methylcholesta-5,*trans*-22-dien-3β-ol, **2**) [3]. More recently, the presence of **2** in the seeds of *B. juncea* was unambiguously proved by 400 MHz ^1H NMR spectroscopy after isolation by means of reverse-phase HPLC [4]. Expecting the widespread occurrence of **2**, accompanying **1** in the seeds of Cruciferae that contain 24-methyl-$\Delta^{5,22}$-sterols, we have undertaken a further investigation by HPLC of the 24-methyl-$\Delta^{5,22}$-sterol fractions isolated from three seed oil samples of *Brassica* sp. and 24 seed samples, including those previously examined by ^{13}C NMR [3], of the following Cruciferae: *Brassica campestris*, *B. hirta*, *B. juncea*, *B. oleracea*, *B. napus* and *Raphanus sativus*.

The acetylated 24-methyl-$\Delta^{5,22}$-sterol fractions, separated from the unsaponifiable lipids of the seed and seed oil samples of Cruciferae in the same manner as described previously [3], showed RR_t 1.10 (cholesteryl acetate, RR_t 1.00) on GLC (OV-1 glsss capillary column). This is consistent with the RR_t of the **1**- and **2**-acetates [4]. The steryl acetates were saponified and the resulting free sterol fractions were subjected to reverse-phase HPLC. The sterol fraction from each of the Cruciferae samples was separated into two well-resolved component peaks by HPLC with RR_t values of 0.86 and 0.92 (cholesterol, RR_t 1.00), which are in accord with those of authentic **2** and **1**, respectively. Thus, the two sterols isolated by HPLC could be identified as brassicasterol (**1**) and 22-dehydrocampesterol (**2**). Identification of these sterols was substantiated by their mass spectra, and further by the

Table 1. Content of C-24 epimeric 24-methyl-$\Delta^{5,22}$-sterols (1 and 2) and their relative abundance in the sterol fractions from the seeds of some *Brassica* and *Raphanus* species of Cruciferae

Seeds and seed oils	24-Methyl-$\Delta^{5,22}$-sterol (%)* (1 and 2)	Relative abundance (%)†	
		1 (24β)	2 (24α)
Brassica campestris var. *chinensis* (Chinese mustard)	13.0	72	28
B. campestris var. *japonica* (potherb mustard)	14.5	78	22
B. campestris var. *pekinensis* (Chinese cabbage)	15.0	76	24
B. campestris var. *periviridis* (tendergreen)	13.5	77	23
B. campestris var. *rapifera* (turnip)	13.5	83	17
B. campestris (candle, A)‡	11.4	82	18
B. campestris (candle, B)‡	11.0	85	15
B. campestris (torch)‡	10.5	84	16
B. hirta var. *alba* (yellow mustard)‡	5.0	92	8
B. juncea var. *cernua* (leaf mustard)	9.0	80	20
B. juncea var. *integrifolia* (Indian mustard)	13.6	69	31
B. juncea (brown mustard)‡	12.6	65	35
B. juncea (oriental mustard)‡§	11.0	68	32
B. oleracea var. *acephala* (kale)	9.9	80	20
B. oleracea var. *alboglabra* (Chinese kale)	14.3	82	18
B. oleracea var. *botrytis* (cauliflower)	9.7	63	37
B. oleracea var. *capitata* (cabbage)	11.3	84	16
B. oleracea var. *gemmifera* (Brussels sprouts)	11.1	71	29
B. oleracea var. *gongylodes* (kohlrabi)	14.2	75	25
B. oleracea var. *italica* (Italian broccoli)	13.9	59	41
B. napus (tower)‡	10.0	85	15
B. napus (midas)‡	11.2	82	18
Brassica sp. (rapeseed oil, China)‖	11.4	72	28
Brassica sp. (rapeseed oil, East Germany)‖	11.1	82	18
Brassica sp. (rapeseed oil, Japan)‖	10.3	84	16
Raphanus sativus (radish)	6.0	81	19
R. sativus var. *longipinnatus* (Japanese radish)	7.6	87	13

* Percentage of 24-methyl-$\Delta^{5,22}$-sterol (1 and 2) in the total sterols determined by GC as the acetate derivative.
† Relative abundance (%) of 1 and 2 in their mixture determined by HPLC as the free sterol.
‡ Cf. ref. [3]. These seeds are used for the commercial production of rapeseed oil.
§ Cf. ref. [4].
‖ Commercially prepared rapeseed oil. Unknown species.

^{13}C NMR spectra [3] for those from the seeds of *B. campestris* (candle and torch), *B. hirta*, *B. juncea* and *B. napus* and, moreover, by 400 MHz ^{1}H NMR [4] for the *B. juncea* (oriental mustard) sterols.

The percentage compositions of the 24-methyl-$\Delta^{5,22}$-sterol fractions (mixture of 1 and 2) in the total sterols of the 27 Cruciferae samples, which were determined by GC of the acetate derivatives, is shown in Table 1, accompanied with the relative abundance of 1 and 2 in the mixture determined by HPLC of the free sterols. As is evident from Table 1, all of the 24-methyl-$\Delta^{5,22}$-sterol fractions isolated from the 27 seed and seed oil samples of *Brassica* and *Raphanus* species were found to contain the 24α-epimer (2), in the range of *ca* 10–40%, in addition to its 24β-epimer (1). This may suggest the ubiquity of the co-occurrence of C-24 epimeric sterols, 1 and 2, in the Cruciferae seeds that contain 24-methyl-$\Delta^{5,22}$-sterols. It might be worth recalling here that the co-occurrence of two other C-24 epimers, 22-dihydrobrassicasterol (24β-methylcholest-5-en-3β-ol) and campesterol (24α-methylcholest-5-en-3β-ol), in higher plants has been observed [5–7].

EXPERIMENTAL

The following seeds, used for the commercial production of rapeseed oil, are of Canadian origin [3]: *Brassica campestris*, 'candle' A and B (different hervest years) and 'torch'; *B. hirta* var. *alba*, 'yellow' mustard; *B. juncea*, 'brown' and 'oriental' mustard; and *B. napus*, 'tower' and 'midas'. Three commercially prepared rapeseed oils from *Brassica* sp. (unknown species) were courteously donated by Dr. I. Niiya, Japan Institute of Oils, Fats and Other Food Inspection, Tokyo. The other seeds of crucifer garden vegetables investigated were generously supplied by Sakata Seeds, Yokohama. Brassicasterol (1) and 22-dehydrocampesterol (2) were used as reference specimens [4].

HPLC was carried out on a Partisil 5 ODS-2 column (Whatman, 25 cm × 8 mm i.d.; packed by Erma Optical Works, Tokyo), using a Kyowa Seimitsu double-plunger pump and a UV detector, monitoring at 210 nm (mobile phase, MeOH–H_2O, 98:2; flow rate, 2 ml/min). Other techniques used in this study and some spectral data have been described previously [3, 4].

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3′-METHOXYDIOSPYRIN, A 7-METHYLJUGLONE DIMER FROM *DIOSPYROS MANNII*

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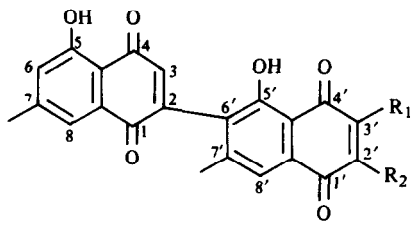
Key Word Index—*Diospyros mannii*; Ebenaceae; 7-methyljuglone dimers; diospyrin; 3′-methoxydiospyrin; triterpenes.

Abstract—Examination of two samples of stem bark of *Diospyros mannii* yielded three naphthoquinones and three triterpenes. Five of the compounds, 7-methyljuglone, diospyrin, lupeol, betulin and betulinic acid, were previously known from other *Diospyros* species and the sixth was characterized as the new naphthoquinone dimer, 3′-methoxydiospyrin.

Diospyros mannii Hiern is a small to medium-sized tree found throughout the rain forest zone of west tropical Africa [1]. In this paper, we report the results of phytochemical analyses of samples of stem bark of this previously uninvestigated species collected in both Cameroon and Ghana and the characterization of a new juglone derivative, 3′-methoxydiospyrin (**3**).

Extraction of the stem bark of material from Cameroon with petrol (bp 40–60°), followed by prep. TLC, gave a wax, the C-2 to C-6′ linked 7-methyljuglone dimer diospyrin (**1**) and the common triterpenes lupeol and betulinic acid. Diospyrin was identified by comparison of physical and spectroscopic data (notably ^{1}H NMR) with that published [2, 3] and the two triterpenes by direct comparison with authentic material. The wax was analysed by GC and EIMS which revealed it to be C_{20}–C_{28} saturated fatty acids (mainly C_{22}, C_{24}, C_{26} and C_{28}) esterified to C_{26} and C_{28} saturated alcohols.

Subsequent extraction with methanol followed by prep. TLC gave further **1** and a yellow-orange compound with mp 220–225°. Accurate mass measurement suggested $C_{23}H_{16}O_7$ and both UV and IR spectra were typical of a juglone derivative. The ^{1}H NMR spectrum showed signals for 7- and 7′-methyl groups, H-6, H-8, H-8′ and H-3, that were in close agreement with those observed for **1**. Of the two hydrogen-bonded hydroxyl protons one, at δ 11.88, agreed closely with that for the 5-hydroxyl group of **1** but the other occurred at δ 12.00 (cf. δ 12.18 for the 5′-hydroxyl in **1**). The two remaining signals, singlets at δ 6.17 (1H) and 3.92 (3H), differed from **1** and must be assigned to a proton and a methoxy substituent which requires this compound to be either 2′-methoxy (**2**) or 3′-methoxydiospyrin (**3**). The EIMS fragmentation confirms that the compound is a benzenoid-quinonoid-linked dimer [4] and agrees with the proposed structures **2** and **3**.



1 $R_1 = R_2 = H$

2 $R_1 = H$, $R_2 = OMe$

3 $R_1 = OMe$, $R_2 = H$

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