Carbon-13 NMR Spectroscopic Analysis of 24-Methyl-$\Delta^{5,22}$-Sterols in Rape and Mustard Seed Oils

T. MATSUMOTO[1], N. SHIMIZU[1], T. ITOH[1], T. IIDA[2] and A. NISHIOKA[3]

ABSTRACT

24-Methylcholesta-5,*E*-22-dien-3β-ol (C_{28} $\Delta^{5,22}$-sterol) was separated from the unsaponifiable matters of the following eight seed oils of Brassica species: *Brassica campestris* (candle I and II and torch), *B. napus* (tower and midas), *B. juncea* (brown and oriental mustards), and *B. alba* (yellow mustard). The configuration at C-24 methyl group of the respective sterols was evaluated by ^{13}C NMR spectroscopy. All the C_{28} $\Delta^{5,22}$-sterols in the Brassica seed oils were found to contain the C-24 epimer of brassicasterol, *trans*-22-dehydrocampesterol, in the range of ca. 10-30%.

INTRODUCTION

The seeds of Cruciferae family are widely used as raw materials for the production of important industrial oils, rape and mustard seed oils (1,2). Since the seed oils contain appreciably large amounts (5-20%) of brassicasterol (24β-methylcholesta-5,*E*-22-dien-3β-ol) in the 4-desmethylsterol fraction of the unsaponifiable matters (USM) (3-8), the occurrence of the sterol has often been utilized for differentiating between Cruciferous oils and other families of seed-bearing oils. Although the structure was tentatively assigned as brassicasterol with a β-methyl group at C-24, the configuration of C-24 seems to be still obscure.

Recent successful application of ^{13}C NMR spectroscopy in both the qualitative and quantitative analysis of C-24 epimeric C-24 alkyl sterols (9) prompted us to examine the C_{28} $\Delta^{5,22}$-sterol present in Brassica seed oils. In this study, the configuration of C-24 of the C_{28} $\Delta^{5,22}$-sterol fraction separated from eight rape and mustard seed oils was analyzed by ^{13}C NMR spectroscopy.

[1]College of Science and Technology, Nihon University, 1-8 Kanda Surugadai, Chiyoda-ku, Tokyo, 101 Japan.
[2]College of Engineering, Nihon University, Koriyama, Fukushima-ken, 963 Japan.
[3]Oil and Fat Research Laboratories, Ajinomoto Co. Inc., Tsurumi-ku, Yokohama, 230 Japan.

EXPERIMENTAL PROCEDURES

Melting points (mp) were uncorrected and infrared (IR) spectra were recorded in KBr pellets. ^1H NMR spectra were obtained at 90 MHz in $CDCl_3$ solution containing tetramethylsilane (TMS) as an internal reference standard. Chemical shifts were given in δ-ppm relative to TMS. Mass spectra (MS, 70 eV) were obtained with a combined gas chromatograph-mass spectrometer (GC-MS, 2m × 3mm id glass column packed with 2% OV-17 on Gas Chrom Z). Gas liquid chromatography (GLC) was carried out on OV-17 SCOT glass capillary column with a flame ionization detector (30m × 0.3mm id; column temperature, 260 C; split ratio, 100:1). Relative retention times (RRT) on the GLC of the acetates of C_{28} $\Delta^{5,22}$-sterols were expressed relative to cholesteryl acetate.

Materials

Seed and USM samples used in this study were as follows: *Brassica napus*, "tower" and "midas"; *B. campestris*, "candle" I and II (different harvest years) and "torch"; *B. juncea*, "brown" and "oriental" mustards; *B. alba*, "yellow" mustard. These samples were imported from Canada. Pure brassicasterol as an authentic specimen was courteously supplied from Dr. H.W. Kircher, College of Agriculture, University of Arizona.

Separation of C_{28} $\Delta^{5,22}$-sterol

C_{28} $\Delta^{5,22}$-sterol fraction was separated from the respective seeds and USM by the following procedure, exemplified by "candle" I.

Extraction of the dried and ground seeds of "candle" I (107 g) by CH_2Cl_2 using Soxhlet apparatus gave a seed oil (46.3 g). The USM (440 mg) extracted from the oil after saponification was fractionated by preparative thin layer chromatography (PLC) on silica gel using hexane-ethyl ether (4:1, v/v) as the developing solvent, to give the 4,4-dimethylsterol, 4-monomethylsterol and 4-desmethylsterol fractions, as described previously (8). The free 4-desmethyl-

TABLE I

Content of USM, Yield of 4-Desmethylsterol Fraction, and Content of C_{28} $\Delta^{5,22}$-Sterol in Eight Brassica Seed Oils

Oil	USM (%)	4-Desmethylsterol (%)[a]	C_{28} $\Delta^{5,22}$-sterol (%)[b]
Tower[c]	–	55.2	10.0
Midas	0.88	56.9	11.2
Candle I	0.95	53.5	11.4
Candle II[c]	–	57.5	11.0
Torch	0.92	51.1	10.5
Brown mustard	0.91	56.7	12.6
Oriental mustard	0.95	52.2	11.0
Yellow mustard	0.87	61.1	5.0

[a]Values were obtained from the USM by PLC.
[b]Values were obtained from the 4-desmethylsterol fraction by glass capillary GLC (see Experimental) as the acetate derivative.
[c]The sample was supplied as the USM.

TABLE II

^{13}C NMR Data for C_{28} $\Delta^{5,22}$-Sterol Separated from Brown Mustard[a] as the Acetate Derivative

Carbon	Brassicasteryl acetate[b]			
C-1	37.0		37.0	
C-2	27.7		27.7	
C-3	73.8		73.8	
C-4	38.0		38.0	
C-5	139.5		139.5	
C-6	122.5		122.5	
C-7	31.8		31.8	
C-8	31.8		31.8	
C-9	50.0		50.0	
C-10	36.5		36.5	
C-11	20.9		20.9	
C-12	39.6		39.6	
C-13	42.2		42.2	
C-14	56.7		56.7	
C-15	24.2		24.2	
C-16	28.4	28.7		28.4
C-17	55.9		55.9	
C-18	12.0		12.0	
C-19	19.2		19.2	
C-20	40.0		40.0	
C-21	20.9		21.0	
C-22	135.7	135.9		135.7
C-23	131.7		131.7	
C-24	42.8	43.0		42.7
C-25	33.1	33.2		33.0
C-26	19.9	19.6		19.9
C-27	19.6	20.1		19.6
C-28	17.6	17.9		17.6
$\underline{C}H_3CO$	20.9		20.9	
Me$\underline{C}$O	170.2		170.2	

[a]In ppm downfield from TMS.
[b]Authentic sample.

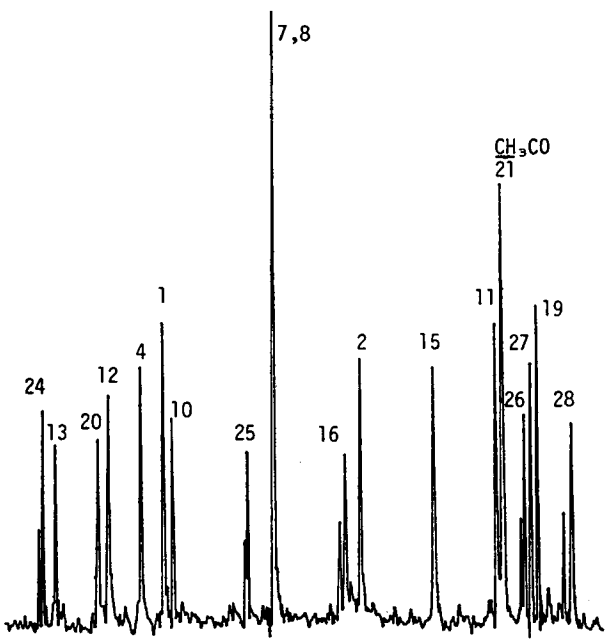

FIG. 1. Partial proton noise-decoupled ^{13}C NMR spectrum of C_{28} $\Delta^{5,22}$-steryl acetate separated from brown mustard. Chemical shift values of individual carbons are shown in Table II and in the text.

sterol fraction (233 mg) was acetylated in acetic anhydride-pyridine overnight at room temperature and the acetate fraction (240 mg) obtained was separated into 3 zones by $AgNO_3$-impregnated silica gel (1:4, w/w) PLC by developing four to six times with CCl_4-CH_2Cl_2 (5:1, v/v). The middle zone with the GLC retention time of 1.14 and the Rf value (relative to cholesteryl acetate) of 0.69 was scratched up and then extracted with ethyl ether to afford the desired C_{28} $\Delta^{5,22}$-steryl acetate (25 mg): mp 154-156 C.

Essentially identical procedure mentioned above was used to separate C_{28} $\Delta^{5,22}$-steryl acetate fractions from the other seed and USM samples.

TABLE III

Relative Intensities (%) of Carbon Signals in the ^{13}C NMR Spectra of C-24 Epimeric C_{28} $\Delta^{5,22}$-Steryl Acetates

Oil		C-16	C-22	C-24	C-25	C-28	Mean
Tower	α	15	14	10	18	17	15
	β	85	86	90	82	83	85
Midas	α	20	17	17	22	22	20
	β	80	83	83	78	78	80
Candle I	α	20	18	19	23	22	20
	β	80	82	81	77	78	80
Candle II	α	18	14	13	17	15	15
	β	82	86	87	83	85	85
Torch	α	22	18	18	15	19	18
	β	78	82	82	85	81	82
Brown mustard	α	37	32	29	32	36	33
	β	63	68	71	68	64	67
Oriental mustard	α	34	31	28	27	34	31
	β	66	69	72	73	66	69
Yellow mustard	α	6	5	9	11	10	8
	β	94	95	91	89	90	92

^{13}C NMR Spectra

The ^{13}C NMR spectra were recorded at 22.53 MHz using a JEOL FX-90Q Fourier transform spectrometer. Conditions were as follows: lock, internal; temperature, 25 C; pulse width, 9 μsec.; flip angle, 45°; spectral width, 5000 Hz; pulse repetition time, 1.0 sec; number of data points, 4096. The samples (ca. 5-6 mg) were each dissolved in $CDCl_3$ (40 μL) containing TMS as an internal reference and measured in 1.7 mm od micro sample tube. Chemical shifts were expressed in δ-ppm relative to TMS. All the spectra were recorded in the proton noise-decoupling mode.

RESULTS AND DISCUSSION

C_{28} $\Delta^{5,22}$-steryl acetate fraction from eight Brassica seed oils was separated from each of the USM by PLC and obtained in the amounts shown in Table I. Although those GLC, IR, ^1H NMR (90 MHz) and MS data were essentially identical to those of an authentic brassicasteryl acetate and with literature values of brassicasteryl acetate isolated from other Cruciferous oils (3-8), no information was obtained concerning the configuration at C-24 from the data: i.e., ("candle" I) $IR_{\nu max}cm^{-1}$, 3030, 960, 825, 823, 797, 1725 and 1260; ^1H NMR δ, 0.70 (3H, *s*, C-18), 1.02 (3H, *s*, C-19), 2.03 (3H, *s*, C-3β-OAc), 0.82 (3H, *d*, *J*=6 Hz, C-27), 0.83 (3H, *d*, *J*=6 Hz, C-26), 0.91 (3H, *d*, *J*=7 Hz, C-28), 1.01 (3H, *d*, *J*=6 Hz, C-21), 5.11 (2H, *m*, C-22, C-23), 4.55 (1H, *bm*, $W_{1/2}$=20 Hz, C-3α) and 5.30 (1H, *m*, C-6); MS m/z (rel int, %), 380 [M^+-AcOH] (100), 365 (11), 337 (9), 296 (8), 282 (10), 255 (30), 253 (11), 228 (11), 213 (15).

Table II shows the ^{13}C NMR chemical shifts of the carbon signals arising from the C_{28} $\Delta^{5,22}$-steryl acetate separated from brown mustard and authentic brassicasteryl acetate. Each signal was assigned by direct comparison with those of the C-24 epimeric 24-methylcholesta-5,*E*-22-dien-3β-ols reported by Wright et al. (9). As can be seen in Table II, signals occurred at 28.4, 135.7, 42.7, 33.0, and 17.6 ppm were assigned to the carbons, C-16, C-22, C-24, C-25, and C-28, respectively, in brassicasteryl acetate with a 24β-

methyl group. Of particular interest was that, in addition to these signals, the spectrum accompany relatively weak signals at slightly lower field (0.1-0.4 ppm) of 28.7, 135.9, 43.0, 33.2, and 17.9 ppm (see Fig. 1) and the chemical shifts were in complete accord with the corresponding signals for the 24α-methyl epimer, *trans*-22-dehydrocampesterol acetate (9). The ^{13}C NMR spectra for the C_{28} $\Delta^{5,22}$-steryl acetates separated from 7 different seed oils were also basically the same as that from brown mustard. It is noticed here that, although the chemical shift differences of the C-26 and C-27 carbons between the C-24 epimeric pair were also observed in each spectrum, the C-27 signal in 24β-isomer and the C-26 signal in 24α-counterpart overlapped each other. All the C_{28} $\Delta^{5,22}$-steryl acetates separated from eight Brassica seed oils were, therefore, regarded as a C-24 epimeric mixture.

Table III shows the relative intensities of the C-16, C-22, C-24, C-25, and C-28 signals in the mixtures. All the signals in a mixture under consideration had similar relative intensities, thus suggesting approximate proportion of the epimers. The intensity measurements established that the C_{28} $\Delta^{5,22}$-sterol fractions in Brassica oils contain ca. 10-30% of *trans*-22-dehydrocampesterol along with brassicasterol.

Nes et al. (10,11) have recently shown by 220 MHz ^{1}H NMR that 24-methylcholesterol fractions (C_{28} Δ^5-sterols) separated from a series of Tracheophytes are always C-24 epimeric mixture with the 24α-isomer presents in about twice the concentration of the 24β-isomer. The present study also demonstrated the co-occurrence of C-24 epimeric 24-methylcholesta-5,*E*-22-dien-3β-ols by ^{13}C NMR spectroscopy, though the 24β-epimer much predominant.

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An Investvation of the Extraction, Refining and Composition of Oil from Winged Bean (*Psophocarpus tetragonolobus* [L.] D.C.)

D. BODGER, J. B. DAVIS, D. FARMERY[1], T. W. HAMMONDS, A. J. HARPER[1], R. V. HARRIS, L. HEBB[1], N. MacFARLANE, P. SHANKS[1] and K. SOUTHWELL,
Tropical Products Institute, 56/62 Gray's Inn Road, London WC1X 8LU, England; and [1]Simon-Rosedowns Ltd., Cannon Street, Hull, England

ABSTRACT

Eleven winged bean accessions from Thailand were analyzed. Oil content ranged between 15 and 18%. Oleic and linoleic acids were the major fatty acids (62.5-64.5%) together with behenic (12.6-14.4%) and lignoceric acid (2.4-2.8%). Linolenic acid level was low and traces of 15-, 17- and 21-carbon acids (saturated and unsaturated) were found. No parinaric acid was detected. Campesterol, stigmasterol and β-sitosterol were the principal components of the unsaponifiable fraction. The extracted oil had a very low free fatty acid (FFA) content but was not completely liquid below 35 C. The refining of crude winged bean oil is reported. Oil produced by expeller had a strong, beany aroma but a negligible level of gums and a low level of FFA. Degumming and neutralizing were unnecessary; bleaching produced an attractive colored oil free from beany aroma. Crude solvent-extracted oils from whole and decorticated winged beans had appreciable contents of gums and higher FFA contents than expeller-produced oil. Laboratory refining demonstrated the strong interference on bleaching exerted by gums and FFA. Conventional refining by degumming, neutralizing, bleaching and deodorizing, and by physical refining produced high-quality oils having a good color, low FFA level and no taste or smell. The solid/liquid ratio of refined winged bean oil as a function of temperature was found to be unusual. Oil was extracted from whole and decorticated winged beans in a pilot solvent extraction plant designed to simulate a Rotocel. Winged bean flakes were not as mechanically strong as those from soybean but good oil extraction yields were obtained and a meal was produced having an oil content of less than 1% at 10% moisture. Whole winged beans were expelled in a small expeller (throughput 16.8 kg/hr). Cake was produced with a residual oil content of 3.3-5% in a single pass through the expeller.

INTRODUCTION

The winged bean (*Psophocarpus tetragonolobus* [L.] D.C.) is a leguminous plant native to tropical Asia and grown there on a small scale for its seeds, immature pods and roots, all of which are used for food. The seeds are rich in protein (30-38%) and also contain oil (15-20%). The oil is said to resemble soybean (1) and groundnut (2) oils.

The potential of the winged bean has been noted (3-6) and it is at present the subject of considerable scientific interest in many tropical countries. The Tropical Products Institute has undertaken a detailed postharvest evaluation of the winged bean and this paper reports findings on the oil content and composition of 11 winged bean accessions from Thailand, extraction of oil by expeller and pilot-scale solvent extraction plant and refining of the oil.