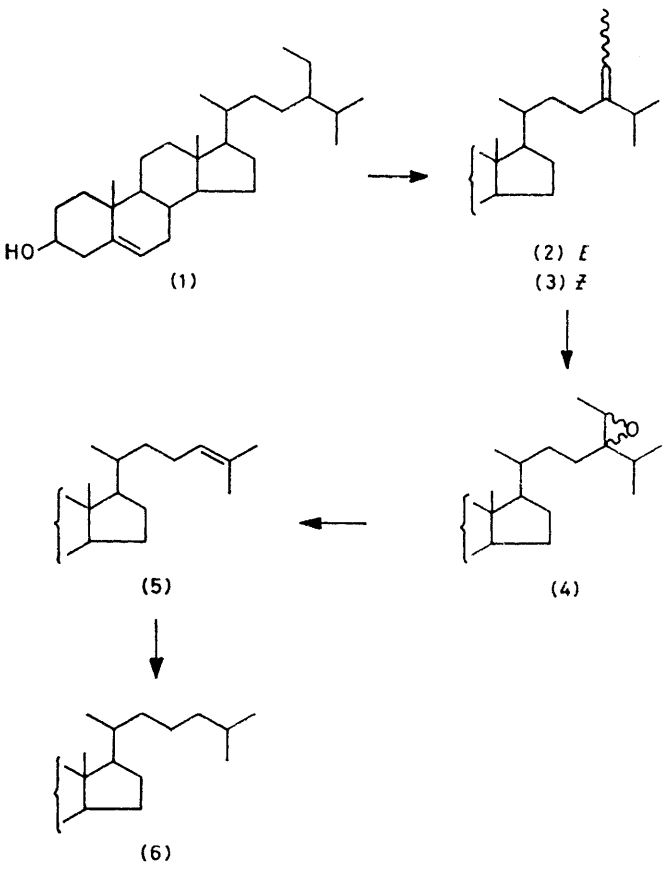
Substrate Stereospecificity in the Metabolism of Fucosterol and Isofucosterol 24,28-Epoxides in *Tenebrio molitor*

By **Francesco Nicotra, Patrizia Pizzi, Fiamma Ronchetti, Giovanni Russo,*** and **Lucio Toma,** Istituto di Chimica Organica dell'Università di Milano, Centro di Studio per le Sostanze Organiche Naturali del C.N.R., Via Saldini 50, 20133 Milano, Italy

It is shown that the insect *Tenebrio molitor* is able to convert the (24*R*,28*S*)-isofucosterol epoxide into cholesterol much better than the (24*S*,28*R*)-isomer; on the other hand, the (24*R*,28*R*)- and the (24*S*,28*S*)-fucosterol epoxides are transformed into cholesterol at about the same extent.

THE metabolism of phytosterols, such as sitosterol (I) by phytophagous insects is generally accepted [1-5] to occur *via* the 24(28)-double-bond compound fucosterol (2), which is subsequently transformed into desmosterol (5) by epoxidation to (4) and elimination of a C-2 unit. The



SCHEME 1

saturation of the 24(25)-double bond of (5) leads to cholesterol (6), which most of the insects need for the biosynthesis of their moulting hormones (Scheme 1).

We have found [6] that in *Tenebrio molitor* the first step in Scheme 1 occurs in a different way, as sitosterol (1) is transformed into both fucosterol (2) and its geometrical isomer isofucosterol (3). We then decided to study the degree of substrate stereospecificity shown by *Tenebrio molitor* in the utilization of the epoxide intermediate (4). To this aim we have synthesized the four diastereoisomeric epoxides (12a), (12b), (14a), and (14b), tritium-labelled at the 7-position, in order to test their utilization by *Tenebrio molitor*.

RESULTS AND DISCUSSION

The synthesis of (24*S*, 28*S*)- and (24*R*, 28*R*)-24,28-epoxystigmast-5-en-3β-ols (5a) and (5b) was effected as follows:† fucosteryl acetate (7) was selectively epoxidized with *m*-chloroperbenzoic acid to give a mixture of the two isomeric epoxides (8a) and (8b). Careful preparative t.l.c. [benzene–ethyl acetate (99 : 1), five elutions] enabled us to avoid the somewhat lengthy procedure of Chen *et al.*,[5] and afforded pure (8a) and (8b). The two isomeric epoxides were practically indistinguishable by ^{1}H n.m.r. spectroscopy, but exhibited different ^{13}C spectra ‡ (see Experimental section).

The absolute configuration of the lower-R_F epoxide (8a) was determined by transforming it into a mixture of the 24-methoxy-28-hydroxy-derivative (9a) and the 24-hydroxy-28-methoxy-derivative (10a) by acid-catalysed methanolysis. These methoxy-alcohols were separated by preparative t.l.c. and their structure was assigned by ^{1}H n.m.r. spectroscopy; the 24-methoxy-28-hydroxy-derivative (9a) [which had the same configuration at C-28 as the starting epoxide (8a)] was submitted to the gas-chromatographic modification of Horeau's method [8] for the determination of the absolute configuration of secondary alcohols (see Table 1). In this way, the lower-R_F epoxide (8a) was established to have the *S*-configuration at C-28.

Analogously, the methanolysis of the higher-R_F epoxide (8b) yielded the methoxy-alcohols (9b) and (10b); again, the application of Horeau's procedure [8] to the 24-methoxy-28-hydroxy-compound (9b) (see Table 1) allowed us to assign to the higher-R_F epoxide (8b) the *R* configuration at C-28.

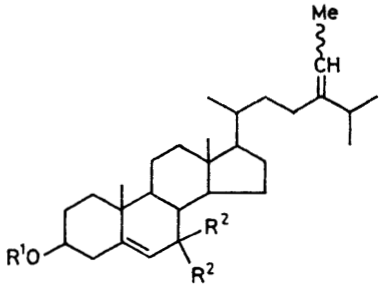
† The synthesis of the diastereoisomeric fucosterol 24,28-epoxides (5a) and (5b) has been reported, without physical data allowing the identification of the individual epoxides, by Chen *et al.*[5] Recently a revision of the previously assigned configurations has been published.[7]

‡ The ^{13}C chemical shifts values are in agreement with those reported in the spectra of the (24*S*,28*S*)- and (24*R*,28*R*)-epoxides kindly sent to us by Professor K. Nakanishi.

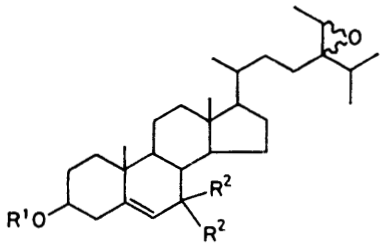
The free epoxy-sterols (5a) and (5b) were obtained by alkaline hydrolysis of the corresponding 3-acetates (8a) and (8b).

The above procedure was repeated to introduce the label: from [7-$^3H_2$]fucosteryl acetate (11) (synthesized according to Knapp *et al.*[9]) [7-$^3H_2$]-(24*S*, 28*S*)- and [7-$^3H_2$]-(24*R*,28*R*)-24,28-epoxystigmast-5-en-3β-ols (12a) and (12b) were obtained. Analogously, [7-$^3H_2$]-(24*R*,-28*S*)- and [7-$^3H_2$]-(24*S*,28*R*)-24,28-epoxystigmast-5-en-3β-ols (14a) and (14b) were synthesized according to Nicotra *et al.*[10] [who describe the synthesis of (6a) and (6b)], starting from [7-$^3H_2$]isofucosteryl acetate (13).[9]

Each of the four tritium-labelled epoxides was mixed with [4-^{14}C]sitosterol and fed, in separate experiments, to young *Tenebrio molitor* larvae. From the acetylated sterol fraction of each experiment a mixture of cholesteryl acetate and sitosteryl acetate was obtained by preparative t.l.c. on silica gel. The two compounds were separated by preparative g.l.c. (2.5% SE-30, T_c = 220 °C) and the pure cholesteryl acetate obtained was diluted with cold material and crystallized to constant specific activity (see Table 2).



(7): E; R^1 = Ac; R^2 = H
(11): E; R^1 = Ac; R^2 = ^{3}H
(13) Z; R^1 = Ac; R^2 = ^{3}H



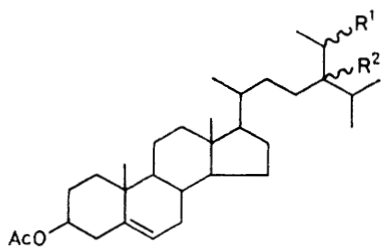
(5a): (24*S*, 28*S*); R^1 = R^2 = H
(5b): (24*R*, 28*R*); R^1 = R^2 = H
(6a): (24*R*, 28*S*); R^1 = R^2 = H
(6b): (24*S*, 28*R*); R^1 = R^2 = H
(8a): (24*S*, 28*S*); R^1 = Ac; R^2 = H
(8b): (24*R*, 28*R*); R^1 = Ac; R^2 = H
(12a): (24*S*, 28*S*); R^1 = H; R^2 = ^{3}H
(12b): (24*R*, 28*R*); R^1 = H; R^2 = ^{3}H
(14a): (24*R*, 28*S*); R^1 = H; R^2 = ^{3}H
(14b): (24*S*, 28*R*); R^1 = H; R^2 = ^{3}H
(15a): (24*S*, 28*S*); R^1 = Ac; R^2 = ^{3}H
(15b): (24*R*, 28*R*); R^1 = Ac; R^2 = ^{3}H
(16a): (24*R*, 28*S*); R^1 = Ac; R^2 = ^{3}H
(16b): (24*S*, 28*R*); R^1 = Ac; R^2 = ^{3}H

TABLE 1

G.l.c detection of (+)-α-phenylethylamides of (−)-*R*- and (+)-*S*-α-phenylbutyric acid representing unreacted anhydride from Horeau's reaction, and determination of the absolute configuration of the secondary alcohols.

Analysed sample	Peak increment (%) *		Absolute configuration at C-28
	R-acid	*S*-acid	
(9b)	−2.7	+2.7	*R*
(9a)	+4.2	−4.2	*S*

* Peak increments are calculated with respect to a parallel blank reaction carried out with cyclohexanol.



(9a): (28*S*); R^1 = OH; R^2 = OMe
(9b): (28*R*); R^1 = OH; R^2 = OMe
(10a): (24*S*); R^1 = OMe; R^2 = OH
(10b): (24*R*); R^1 = OMe; R^2 = OH

The ^{3}H : ^{14}C ratios of cholesteryl acetate reported in Table 2 show that the (24*R*,28*S*)-isofucosterol epoxide (14a) is utilized by the insect much better than its (24*S*,-

28*R*)-isomer (14b), while the (24*S*,28*S*)- and the (24*R*,28*R*)-fucosterol epoxides (12a) and (12b) are utilized to about the same extent.

It is interesting to note also that the insect *Bombyx mori* shows a low degree of specificity in the metabolism of the fucosterol epoxides, whereas, in nutritional experiments, the isofucosterol epoxides were unable to support growth and development of silkworm larvae.[11]

Our experiments clearly indicate that the substrate stereospecificity shown by *Tenebrio molitor* is good for the isofucosterol (24,28)-epoxides, but quite low for the fucosterol (24,28)-epoxides.

TABLE 2

Total radioactivities and ^3H : ^{14}C ratios of the administered precursors and of the isolated cholesteryl acetate

Experiment no.	Administered precursors: Compounds	^3H : ^{14}C ratios	Recovered cholesteryl acetate: ^{14}C (d.p.m.)	^3H : ^{14}C ratios
1	[7-$^3H_2$]-(24*S*,28*S*)-24,28-epoxystigmast-5-en-3β-ol (12a) + [4-^{14}C]-sitosterol (6.35×10^6 d.p.m. of ^{14}C)	8.4	3.25×10^5	6.8 : 1
2	[7-$^3H_2$]-(24*R*,28*R*)-24,28-epoxystigmast-5-en-3β-ol (12b) + [4-^{14}C]-sitosterol (5.34×10^6 d.p.m. of ^{14}C)	9.3	3.86×10^5	10.9 : 1
3	[7-$^3H_2$]-(24*R*,28*S*)-24,28-epoxystigmast-5-en-3β-ol (14a) + [4-^{14}C]-sitosterol (6.36×10^6 d.p.m. of ^{14}C)	19.3	2.18×10^5	35.3 : 1
4	[7-$^3H_2$]-(24*S*,28*R*)-24,28-epoxystigmast-5-en-3β-ol (14b) + [4-^{14}C]-sitosterol (9.14×10^6 d.p.m. of ^{14}C)	14.6	3.19×10^5	2.5 : 1

EXPERIMENTAL

All m.p.s are uncorrected. Optical rotations were obtained using a Perkin-Elmer 141 polarimeter for chloroform solutions. Elemental analyses were consistent with the calculated values. ^1H N.m.r. and ^{13}C n.m.r. spectra were recorded on a Varian XL-100 spectrometer in deuteriochloroform solutions. Gas-liquid chromatography (g.l.c.) was carried out with a Carlo-Erba Fractovap 2400 V instrument on 2-m columns packed with 1% OV-17 at 215 °C or 2.5% SE-30 at 220 °C. Preparative and analytical t.l.c. was carried out on Riedel-De Haen A.g. DC-SI silica gel plates; the products were detected by spraying with 50% aqueous sulphuric acid and heating at 110 °C for 5 min. 'Usual work-up' refers to dilution with water, extraction with an organic solvent, washing to neutrality, drying over Na_2SO_4, filtration, and evaporation *in vacuo*. Radioactive samples were counted on a Packard Tri-Carb 3320 liquid-scintillation counter; the samples were dissolved into 10 ml of a solution consisting of 0.65% (w/v) 2,5-diphenyloxazole and 0.013% (w/v) 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene in toluene–dioxan (1 : 1, v/v).

(24S,28S)- *and* (24R,28R)-3β-*Acetoxy*-24,28-*epoxystigmast*-5-*enes* (8a) *and* (8b).—To a solution of fucosteryl acetate (7) (300 mg) in $CHCl_3$ (20 ml) a solution of *m*-chloroperbenzoic acid (150 mg) (30% excess) in $CHCl_3$ (20 ml) was added at 0 °C. The mixture was stirred for 10 min and then quenched by addition of 50% aqueous $FeSO_4$ (20 ml). Usual work-up afforded crude product (307 mg), which was chromatographed on silica gel–Celite (1 : 1). By elution with benzene a mixture (115 mg) of (8a) and (8b) was recovered, which was carefully separated by preparative t.l.c [benzene–ethyl acetate (99 : 1), five elutions].

The lower-R_F epoxide, (24*S*,28*S*)-3β-acetoxy-24,28-epoxystigmast-5-ene (8a) (65 mg) was crystallized from diethyl ether–methanol, m.p. 94—95 °C; $[\alpha]_D^{20}$ −44.0; δ_H 0.67 (s, 18-Me), 0.91 (d, *J* 6 Hz, 26- or 27-Me), 0.93 (d, *J* 6 Hz, 26- or 27-Me), 0.98 (d, *J* 6 Hz, 21-Me), 1.00 (s, 19-Me), 1.27 (d, *J* 6 Hz, 29-Me), 2.04 (s, $MeCO_2$), 2.95 (q, *J* 6 Hz, 28-CH), 4.6 (m, 3-CH), and 5.4 (m, 6-CH); δ_C 170.20 (C=O), 139.56 (C-5), 122.47 (C-6), 73.87 (C-3), 66.09 (C-24), 56.67 (C-28 and C-14), 55.61 (C-17), 50.04 (C-9), 42.31 (C-4), 39.71 (C-16), 38.13 (C-4), 37.00 (C-1), 36.59 (C-10), 36.05 (C-20), 32.48 (C-25), 31.88 (C-7, C-8), 31.59 (C-22), 28.21 (C-12), 27.78 (C-2), 25.24 (C-23), 24.28 (C-15), 21.32 (*Me*CO_2), 21.03 (C-11), 19.28 (C-19), 18.71 (C-26 or C-27), 18.19 (C-26, or C-27), 17.98 (C-21), 14.27 (C-29), and 11.84 (C-18).

The higher-R_F epoxide, (24*R*,28*R*)-3β-acetoxy-24,28-epoxystigmast-5-ene (8b) (75 mg) was crystallized from diethyl ether–methanol, m.p. 113—115 °C; $[\alpha]_D^{20}$ −44.9; δ_H 0.67 (s, 18-Me), 0.88 (d, *J* 6 Hz, 26- or 27-Me), 0.92 (d, *J* 6 Hz, 26- or 27-Me), 0.99 (d, *J* 6 Hz, 21-Me), 1.01 (s, 19-Me), 1.29 (d, *J* 6 Hz, 29-Me), 2.04 (s, $MeCO_2$), 2.95 (q, *J* 6 Hz, 28-CH), 4.6 (m, 3-CH), and 5.4 (m, 6-CH); δ_C 170.19 (C=O), 139.61 (C-5), 122.53 (C-6), 73.93 (C-3), 66.04 (C-24), 56.32 (C-28), 56.76 (C-14), 55.98 (C-17), 50.11 (C-9), 42.41 (C-4), 39.82 (C-16), 38.19 (C-4), 37.09 (C-1), 36.65 (C-10), 36.30 (C-20), 32.14 (C-25), 31.94 (C-7, C-8), 31.34 (C-22), 28.30 (C-12), 27.86 (C-2), 25.54 (C-23), 24.34 (C-15), 21.31 (*Me*CO_2), 21.11 (C-11), 19.33 (C-19), 18.68 (C-26 or C-27), 18.48 (C-26 or C-27), 17.79 (C-21), 14.28 (C-29), and 11.88 (C-18).

Methanolysis of Epoxides (8a) *and* (8b).—The lower-R_F epoxide (8a) (25 mg) was dissolved in 0.02N methanolic H_2SO_4 (3 ml) and refluxed for 10 min. After removal of methanol *in vacuo*, usual work-up afforded 23 mg an 8 : 2 mixture of two products, (9a) and (10a), which were separated by preparative t.l.c using benzene–ethyl acetate (9 : 1) as eluant: (9a) (lower-R_F product, 12.5 mg), m.p. (from diethyl ether) 104—106 °C; δ_H 0.67 (s, 18-Me), 1.01 (s, 19-Me), 1.01 (d, *J* 6 Hz, 21-, 26-, 27-Me), 1.23 (d, *J* 6 Hz, 29-Me), 2.03 (s, $MeCO_2$), 3.27 (s, MeO), 3.9 (q, *J* 6 Hz, 28-CH), 4.6 (m, 3-CH), and 5.4 (m, 6-CH): (10a) (higher-R_F product, 3 mg); δ_H 0.67 (s, 18-Me), 0.86 (d, *J* 6 Hz, 21-Me), 0.93 (d, *J* 6 Hz, 26-, 27-Me), 1.01 (s, 19-Me), 1.13 (d, *J* 6 Hz, 29-Me), 2.03 (s, $MeCO_2$), 3.30 (q, *J* 6 Hz, 28-CH), 3.35 (s, MeO), 4.6 (m, 3-CH), and 5.4 (m, 6-CH). In the same way 25 mg of the higher-R_F epoxide (8b) afforded 22 mg of an 8 : 2 mixture of two products, (9b) and (10b), which were separated by preparative t.l.c as above: (9b) (lower-R_F product, 10 mg), m.p. (from diethyl ether) 118—120 °C; δ_H 0.67 (s, 18-Me), 1.01 and 1.02 (2 × d, *J* 6 Hz, 21-, 26-, 27-Me), 1.01 (s, 19-Me), 1.22 (d, *J* 6 Hz, 29-Me), 2.03 (s, $MeCO_2$), 3.27 (s, MeO), 3.9 (q, *J* 6 Hz, 28-CH), 4.6 (m, 3-CH), and 5.4 (m, CH); (10b) (higher-R_F product, 2.5 mg); δ_H 0.67 (s, 18-Me), 0.86 (d, *J* 6 Hz, 21-Me), 0.93 (d, *J* 6 Hz, 26-, 27-Me), 1.01 (s, 19-Me), 1.13 (d, *J* 6 Hz, 29-Me), 2.03 (s, $MeCO_2$), 3.30 (q, *J* 6 Hz, 28-CH), 3.35 (s, MeO^-), 4.6 (m, 3-CH), and 5.4 (m, 6-CH).

Application of the Gas-chromatographic Modification [8] *of Horeau's Method to* (9a) *and* (9b).—The 24-methoxy-28-alcohols (9a) and (9b) (6.5 mg each) were dissolved in pyridine (10 μl) and treated with of (±)-α-phenylbutyric anhydride (7.5 μl). The reaction mixtures were kept at 40 °C for 1.5 h in a closed vial. A parallel reference reaction was carried out with cyclohexanol. (*R*)-α-Phenylethylamine (9 μl) was then injected and mixed thoroughly by agitation. After 15 min the reaction mixtures were each diluted with dry tetrahydrofuran (1 ml) and submitted to a gas-chromatographic analysis. The areas of the peaks of the amides of (−)-(*R*)- and (+)-(*S*)-α-phenylbutyric acid obtained from (9a) and (9b) were compared to those of cyclohexanol and the increment (or decrement) percentage was assessed (Table 1).

(24S,28S)-24,28-*Epoxystigmast*-5-*en*-3β-*ol* (5a).—Compound (8a) (40 mg) dissolved in 0.25% methanolic KOH (10 ml) was refluxed for 1.5 h. Methanol was removed *in vacuo*; usual work-up afforded (5a) (37 mg) which was crystallized from methanol, m.p. 121—123 °C; $[\alpha]_D^{20}$ −41.6; δ_H 0.67 (s, 18-Me), 0.90 (d, *J* 6 Hz, 26-, 27-Me), 0.96 (d, *J* 6 Hz, 21-Me), 1.00 (s, 19-Me), 1.26 (d, *J* 6 Hz, 29-Me), 2.95 (q, *J* 6 Hz, 28-CH), 3.5 (m, 3-CH), and 5.4 (m, 6-CH).

(24R,28R)-24,28-*Epoxystigmast*-5-*en*-3β-*ol* (5b).—Compound (8b) (50 mg), treated as above, yielded (5b) (46 mg) which was crystallized from methanol, m.p. 160—162 °C; $[\alpha]_D^{20}$ −42.9; δ_H 0.67 (s, 18-Me), 0.87 (d, *J* 6 Hz, 26- or 27-Me), 0.91 (d, *J* 6 Hz, 26- or 27-Me), 0.96 (d, *J* 6 Hz, 21-Me), 1.00 (s, 19-Me), 1.26 (d, *J* 6 Hz, 29-Me), 2.95 (q, *J* 6 Hz, 28-CH), 3.5 (m, 3-CH), and 5.4 (m, 6-CH).

[7-$^3H_2$]-(24S,28S)- *and* (24R,28R)-3β-*Acetoxy*-24,28-*epoxystigmast*-5-*enes* (15a) *and* (15b). To a solution of [7-$^3H_2$]fucosteryl acetate [9] (34 mg) (11) (radiochemically pure by gas chromatography, OV-17, 1%, T_c 260 °C, specific activity 13.5 mCi mmol^{-1}) dissolved in $CHCl_3$ (4 ml), a solution of *m*-chloroperbenzoic acid (17 mg), dissolved in $CHCl_3$ (1 ml), was added. The mixture was stirred at 0 °C, and after 10 min was quenched with a 50% aqueous $FeSO_4$ solution. Usual work-up afforded 35 mg of crude reaction product. The mixture of (15a) and (15b) was separated by careful preparative t.l.c as described for the cold material, to yield 3.5 mg of the lower-R_F epoxide, [7-$^3H_2$]-(24*S*,28*S*)-3β-acetoxy-24,28-epoxystigmast-5-ene (15a) (specific activity 13.8 mCi mmol^{-1}), and 4.3 mg of the higher-R_F epoxide, [7-$^3H_2$]-(24*R*,28*R*)-3β-acetoxy-24,28-epoxystigmast-5-ene (15b) (specific activity 13.8 mCi mmol^{-1}).

[7-$^3H_2$]-(24S,28S)-24,28-*Epoxystigmast*-5-*en*-3β-*ol* (12a).—Compound (15a) (3.5 mg) was dissolved in 0.25% methanolic KOH (10 ml) and refluxed for 1 h. The solvent was removed *in vacuo*; usual work-up afforded a crude product, which was filtered through silica gel using benzene–ethyl acetate (8 : 2); 2 mg of pure (12a) (specific activity 13.8 mCi mmol^{-1}) was obtained.

[7-$^3H_2$]-(24R,28R)-24,28-*Epoxystigmast*-5-*en*-3β-*ol* (12b).—Compound (15b) (4.3 mg), treated as described for (15a), yielded 2 mg of pure (12b) (specific activity 13.8 mCi mmol^{-1}).

[7-$^3H_2$]-(24R,28S)- *and* (24S,28R)-3β-*Acetoxy*-24,28-*epoxystigmast*-5-*enes* (16a) *and* (16b).—[7-$^3H_2$]Isofucosteryl acetate (33 mg) (13) [9] (radiochemically pure by gas-chromatography, OV-17 1%, T_C 260 °C, specific activity 24.5 mCi mmol^{-1}) was treated as described for (11), affording 38 mg of crude product. The mixture of (16a) and (16b) was separated by careful preparative t.l.c as described for the cold material, to yield 3 mg of the lower-R_F epoxide, [7-$^3H_2$]-(24*R*,28*S*)-3β-acetoxy-24,28-epoxystigmast-5-ene (16a) (specific activity 24.8 mCi mmol^{-1}), and 5 mg of higher-R_F epoxide, [7-$^3H_2$]-(24*S*,28*R*)-3β-acetoxy-24,28-epoxystigmast-5-ene, (16b) (specific activity 24.8 mCi mmol^{-1}).

[7-$^3H_2$]-(24*R*,28*S*)-24,28-*Epoxystigmast*-5-*en*-3β-*ol* (14a).—Compound (16a) (3 mg), treated as described for (15a), yielded 2.3 mg of pure (14a) (specific activity 24.8 mCi mmol^{-1}).

[7-$^3H_2$]-(24S,28R)-24,28-*Epoxystigmast*-5-*en*-3β-*ol* (14b).—Compound (16b) (5 mg) treated as described for (15a), yielded 4.2 mg of pure (14b) (specific activity 24.8 mCi mmol^{-1}).

Administration of Labelled Precursors and Isolation of Labelled Cholesteryl Acetate (*Experments* 1, 2, 3, *and* 4).—Each [7-$^3H_2$]-24,28-epoxystigmast-5-en-3β-ol, mixed with [4-^{14}C]sitosterol (Radiochemical Centre, Amersham; the radioactivities and the ^3H : ^{14}C ratios are reported in Table 2), was deposited onto 300 mg of finely grounded oatmeal and fed to 175 young *Tenebrio molitor* larvae, 1—1.5 cm long (3.1 g), after two starving days. Four days later the larvae were sacrificed by freezing, macerated in methanol, and refluxed for 1.5 h with 0.25% methanolic KOH (150 ml). Uusal work-up afforded the non-saponifiable fraction which was acetylated and separated by preparative t.l.c on silica gel [eluant benzene–ethyl acetate (98 : 2)] into two bands. The less-polar band corresponded to the mixture of sitosteryl and cholesteryl acetates, whereas the more-polar one corresponded to the acetate of the unconverted epoxide. Sitosteryl and cholesteryl acetates were separated by preparative g.l.c (2.5% SE-30, T_C 220 °C). The pure cholesteryl acetate obtained in this way was diluted to 50 mg with cold material and crystallized from diethyl ether–methanol to constant specific activity (see Table 2).

We thank Dr. A. Longoni, Centro Ricerche Antiparassitarie Montedison S.p.A, for *Tenebrio molitor* insects, and Dr. G. Severini Ricca for the n.m.r. spectra.

[0/737 *Received*, 19*th May*, 1980]

REFERENCES

[1] J. A. Svoboda, M. J. Thompson, and W. E. Robbins, *Nature New Biol.*, 1971, **230**, 57.
[2] J. P. Allais and M. Barbier, *Experientia*, 1971, **27**, 506.
[3] M. Morisaki, H. Ohtaka, M. Okubayashi, N. Ikekawa, Y. Horie, and S. Nakasone, *J.C.S. Chem. Comm.*, 1972, 1275.
[4] J. P. Allais, A. Alcaide, and M. Barbier, *Experientia*, 1973, **29**, 944.
[5] S.-M. L. Chen, K. Nakanishi, N. Awata, M. Morisaki, N. Ikekawa, and Y. Shimizu, *J. Amer. Chem. Soc.*, 1975, **97**, 5297.
[6] F. Nicotra, F. Ronchetti, and G. Russo, *Experientia*, 1978, **34**, 699.
[7] Y. Fujimoto, K. Murakami, and N. Ikekawa, *J. Org. Chem.*, 1980, **45**, 566.
[8] C. J. W. Brooks and J. D. Gilbert, *J.C.S. Chem. Comm.*, 1973, 194.
[9] F. F. Knapp, L. J. Goad, and T. W. Goodwin, *Phytochemistry*, 1977, **16**, 1683.
[10] F. Nicotra, F. Ronchetti, G. Russo, and L. Toma, *Gazzetta*, 1980, **110**, 151.
[11] Y. Fujimoto, M. Morisaki, and N. Ikekawa, *Biochemistry*, 1980, **19**, 1065.