Studies on Biochemical Transformation of Plant S... oids. Part IV.[1] Biosynthesis of 3α-Hydroxylated Steroidal Sapogenins. Part I

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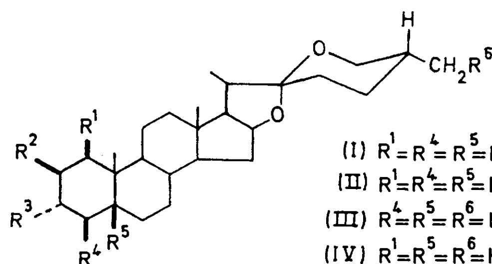
[1-^{3}H]Diosgenin (VI), [1-^{3}H]diosgenone (VII), [1-^{3}H]smilagenone (X), [1-^{3}H]smilagenin (VIII), and [1-^{3}H]-epismilagenin (IX) were incubated with the homogenate from *Dioscorea tokoro* seedlings at 27—29° for 7 h. The results indicate that epismilagenin is a most important precursor in the biosynthesis of 3α-hydroxylated steroidal sapogenins.

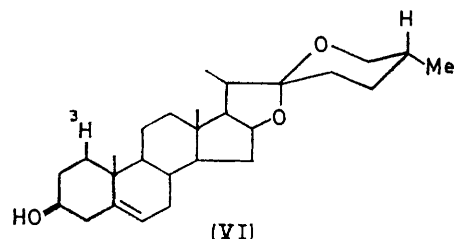
A CHARACTERISTIC feature of the family *Dioscoreaceae* is the existence of steroidal sapogenins having a 3α-hydroxy-group.[2] The following sapogenins have been isolated from *Dioscorea tokoro* Makino: yonogenin [3] (I), igagenin [4] (II), tokorogenin [5] (III), isodiotigenin [6] (IV), and kogagenin [7] (V); they were shown to occur as free sapogenins in the aerial parts of the plant, and as saponins in the underground parts; further, only spirostans containing a 3α-hydroxy-group and a 5β-hydrogen atom were found as free sapogenins in the plant.

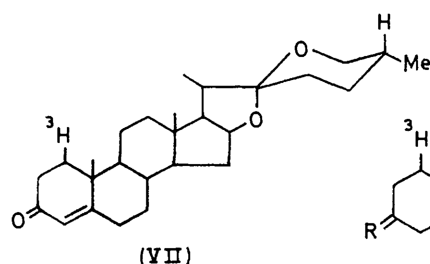
[1] Part III, H. Minato, A. Shimaoka, and K. Takeda, *J. Chem. Soc.* (*C*), 1969, 1483.
[2] K. Takeda, *Progr. Phytochem.*, 1971, **3**, in the press.
[3] K. Takeda, T. Okanishi, and A. Shimaoka, *Chem. and Pharm. Bull.* (*Japan*), 1958, **6**, 532.
[4] F. Yasuda, Y. Nakagawa, A. Akahori, and T. Okanishi, *Tetrahedron*, 1968, **24**, 6535.
[5] M. Nishikawa, K. Morita, H. Hagiwara, and M. Inoue, *J. Pharm. Soc. Japan*, 1954, **74**, 1165; K. Morita, *Bull. Chem. Soc. Japan*, 1959, **32**, 791.
[6] K. Takeda, G. Lukacs, and F. Yasuda, *J. Chem. Soc.* (*C*), 1968, 1041.
[7] K. Takeda, T. Kubota, and A. Shimaoka, *Tetrahedron*, 1959, **7**, 62; T. Kubota, *Chem. and Pharm. Bull.* (*Japan*), 1959, **7**, 898; T. Kubota and K. Takeda, *Tetrahedron*, 1960, **10**, 1.

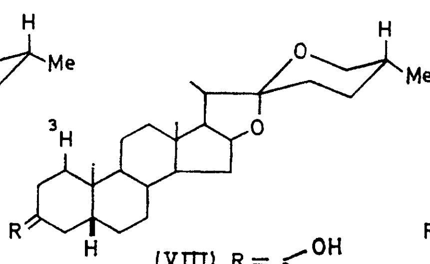
The Kawasaki group has isolated saponins of yonogenin (I) and tokorogenin (III) (yononin [8] and tokoronin [9], respectively) and established their structures. In these compounds, the sugar portion is always attached to a hydroxy-group other than the 3α-hydroxy-group, which remains unsubstituted.

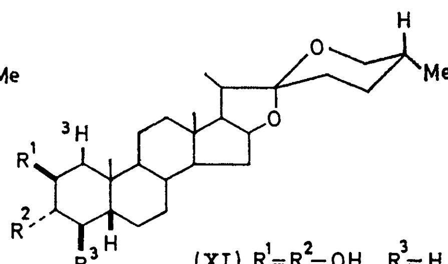
Radioactive compounds were synthesised as follows. [1-^{3}H]Diosgenone (VII) was made by hydrogenation of the 1,4-dien-3-one (XIII) with tritium followed by treatment with an alkali. [1-^{3}H]Diosgenin (VI) was obtained by sodium borohydride reduction of the enol acetate of (VII). When compound (VII) was hydrogenated with 10% palladised charcoal in sodium hydroxide–ethanol, [1-^{3}H]smilagenone (X) was obtained, and reduction of compound (X) with lithium hydrido-tri-t-butoxyaluminate gave [1-^{3}H]smilagenin (VIII) and [1-^{3}H]epismilagenin (IX).

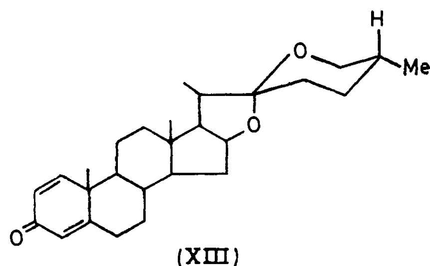


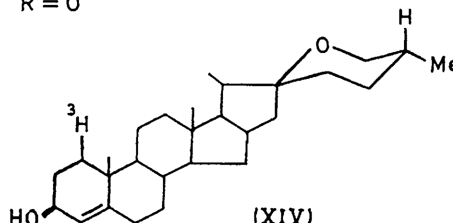












We hoped to clarify the biosynthetic pathway to the 3α-hydroxylated sapogenins, and selected the biosynthesis of yonogenin (I) as an object of study.

Recently, Tomita and Uomori [10,11] established by the use of *Dioscorea tokoro* tissue cultures that conversion of tokorogenin (III) into yonogenin (I) by the route postulated by Marker,[12] *i.e.* by the removal of one hydroxy-group, does not occur. Two biosynthetic routes to the 3α-hydroxylated sapogenin, yonogenin (I), were therefore considered possible; the first involves oxidation of the 3β-hydroxy-group followed by reduction to give the 3α-hydroxy-derivative and the second route oxidation of the 5β-spirost-2-en. The former is discussed in this paper and the latter will be in a subsequent paper.

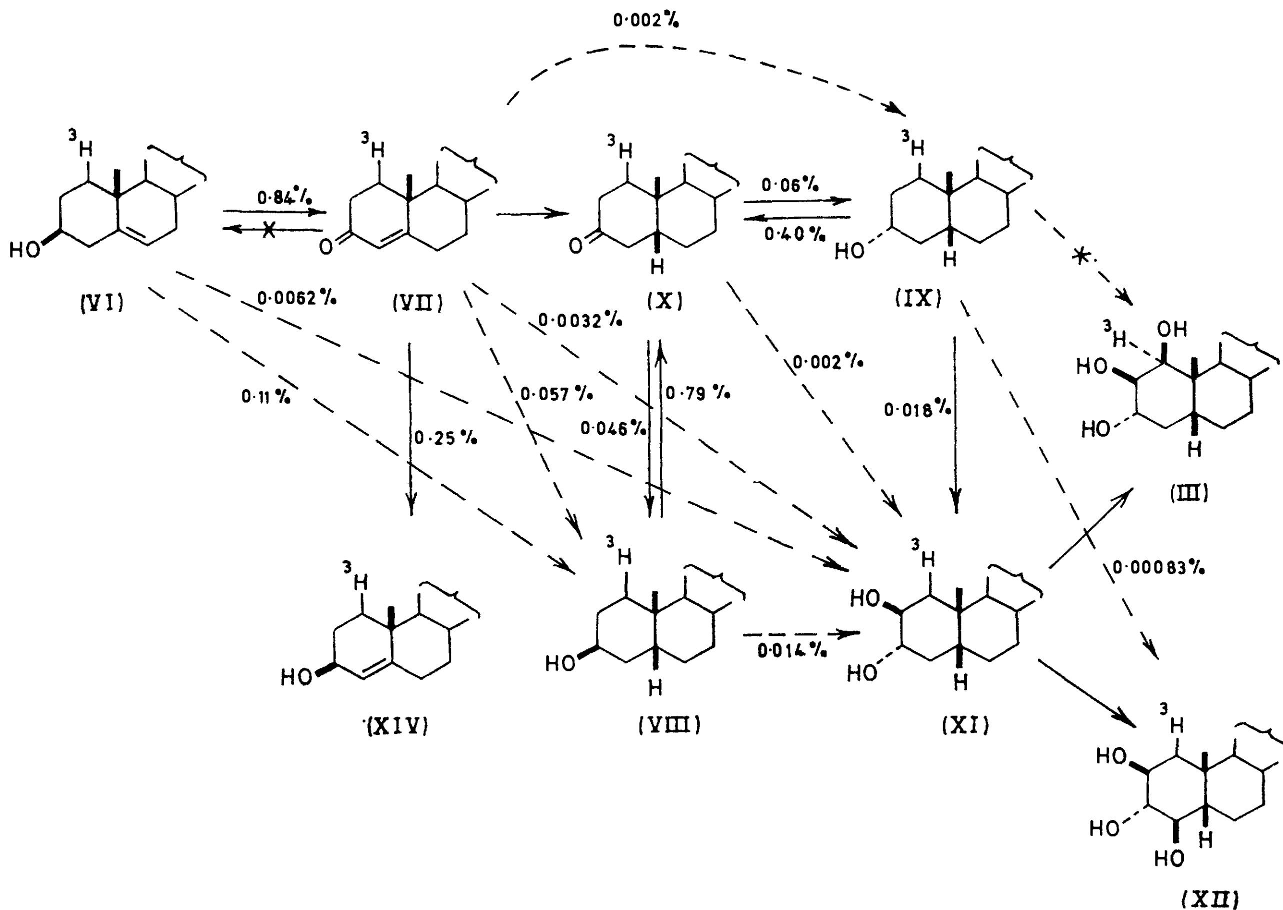
We used a homogenate of *D. tokoro* seedlings, taken about 1 month after germination. Radioactive compounds were added to this homogenate, which was then incubated at 27—29° for 7 h. The mixture was saponified with 5% sulphuric acid in methanol followed by 5% potassium hydroxide in methanol, and extracted with chloroform. The extract was separated by preparative t.l.c. on silica gel. Labelled diosgenin (VI) was converted into diosgenone (VII) and smilagenin (VIII) with incorporations of 0·84 and 0·11%, respectively, together with a small amount of yonogenin (XI) (incorporation 0·0062%). Diosgenone (VII) was converted mainly

[8] T. Kawasaki and K. Miyahara, *Tetrahedron*, 1965, **21**, 3633.
[9] T. Kawasaki and T. Yamauchi, *J. Pharm. Soc. Japan*, 1963, **83**, 757; K. Miyahara and T. Kawasaki, *Chem. and Pharm. Bull.* (*Japan*), 1969, **17**, 1369; K. Miyahara, F. Isozaki, and T. Kawasaki, *ibid.*, p. 1735.
[10] Y. Tomita and A. Uomori, *Chem. Comm.*, 1971, 284.
[11] Y. Tomita and A. Uomori, *Phytochemistry*, to be published.
[12] E. Heftmann, *Lloydia*, 1968, **31**, 293.

into a 3β-hydroxy-Δ^4-derivative (XIV) (0·25%); smilagenin (VIII) (0·057%) and small amounts of epismilagenin (IX) (0·002%) and yonogenin (XI) (0·0032%) were also formed. Smilagenin (VIII) and epismilagenin (IX) were converted into smilagenone (X) (incorporation 0·79 and 0·40%, respectively), and smilagenone (X) was converted into smilagenin, epismilagenin, and yonogenin, though in low yields (incorporation 0·046, 0·06, and 0·002%, respectively).

Smilagenin (VIII) and epismilagenin (IX) also gave yonogenin (XI) (incorporations 0·014 and 0·018%, respectively). Only very slight incorporation (0·00083%) into isodiotigenin (XII) occurred on incubation of epismilagenin. However, in all the other experiments isodiotigenin was isolated but was radioinactive, and in all experiments the tokorogenin * (III) isolated was radioinactive.

The results (Scheme) indicate that smilagenone (X) is an important intermediate in this pathway and epismilagenin (IX) is a most probable precursor in the biosynthesis of 3α-hydroxylated steroidal sapogenins. We could not, however, ascertain whether or not the conversion reactions were occurring *via* the furostanol glycosides [14] (glycosides having a ring-F opened aglycone) because the sapogenins were isolated by acid hydrolysis of the incubation mixtures.



SCHEME —— Probable direct reaction; – – – – reaction involving several steps

* Since [1-^{3}H]diosgenone (VII) is a mixture [13] of [1α-^{3}H]diosgenone and the [1β-^{3}H]-isomer, and all normal steroid hydroxylations proceed with retention of configuration, tokorogenin produced should be radioactive unless a 1-oxo-derivative is involved in this biosynthetic route. In our previous work [2] no 1-oxo-sapogenins were isolated from *D. tokoro*.

EXPERIMENTAL

Radiochemical analyses for specific activities employed a Nuclear–Chicago Liquid Scintillater (720 series) for sapogenins in 15 ml of scintillation fluid [PPO (6 g) and POPOP (100 mg) in toluene (1 l)]. Solvent systems for t.l.c. were (A) n-hexane–chloroform–ethyl acetate (4 : 1 : 1) and (B) benzene–acetone–ethyl acetate–chloroform–methanol (1 : 1 : 1 : 1 : 0·1); R_F values are shown in the Table.

	R_F		R_F
Diosgenin (VI)	0·18 (A)	Acetate	0·57 (A)
Diosgenone (VII)	0·28 (A)		
Smilagenin (VIII)	0·22 (A)	Acetate	0·59 (A)
Epismilagenin (IX)	0·19 (A)	Acetate	0·58 (A)
Smilagenone (X)	0·39 (A)		
Yonogenin (XI)	0·43 (B)	Acetate	0·41 (A)
Isodiotigenin (XII)	0·20 (B)	Acetate	0·24 (A)
Tokorogenin (III)	0·27 (B)	Acetate	0·27 (A)
3β-Acetoxyspirost-4-en	0·57 (A)		

[13] H. J. Brodie, K. Raab, G. Possanza, N. Seto, and M. Gut, *J. Org. Chem.*, 1969, **34**, 2697.
[14] S. Kiyokawa, M. Hutoh, T. Komori, T. Nohara, I. Hosokawa, and T. Kawasaki, *Chem. and Pharm. Bull.* (*Japan*), 1968, **16**, 1162.

[1-^{3}H]*Diosgenone* (VII).—A solution of spirosta-1,4-dien-3-one (XIII) (104 mg) in dioxan (10 ml) was hydrogenated with pure tritium gas (8·45 ml; 1·0 ml ≡ 2·5 Ci) over 5% palladium–charcoal at 25° for 1 day, and unconsumed tritium (5·4 ml) was recovered. The mixture was filtered and evaporated *in vacuo* to leave a crystalline residue (102 mg; 2·8 Ci). As the residue showed three spots (dihydro-derivative, tetrahydro-derivative, and starting material) on t.l.c., it was separated by preparative t.l.c. on silica gel to give [1,2-$^3H_2$]diosgenone (450 mCi). This was dissolved in 3% potassium hydroxide–methanol and heated under reflux for 2 h. The mixture was extracted with chloroform, washed with water, dried (Na_2SO_4), and evaporated, leaving a crystalline residue. The residue was dissolved in methanol (10 ml) and the solution evaporated *in vacuo*. This operation was repeated three times, and the residue was crystallised from ethanol to give [1-^{3}H]*diosgenone* (VII) (260 mCi; 10·7 mg; specific activity 10·0 Ci mmol^{-1}), identical with the authentic sample of diosgenone, m.p. 186—188° (mixed m.p., i.r. spectra, and t.l.c.).

[1-^{3}H]*Diosgenin* (VI).—A mixture of [1-^{3}H]diosgenone (VII) (247 mCi; specific activity 20 Ci mmol^{-1}), carrier diosgenone (20 mg), and toluene-*p*-sulphonic acid (15 mg) in isopropenyl acetate (15 ml) was heated under reflux for 3 h under nitrogen. Sodium acetate (100 mg) was added and the mixture was evaporated *in vacuo*. A solution of the residue in ether–chloroform (1 : 1) was washed with 5% potassium hydrogen carbonate–ice–water and water, dried (Na_2SO_4), and evaporated, leaving a residue (an enol acetate) (245 mCi; 20 mg). This residue was dissolved in 95% ethanol (20 ml) and reduced with sodium borohydride (120 mg) in ethanol (5 ml) at room temperature. Potassium hydroxide (50%; 2 ml) was added and the mixture was stirred for 1 h at room temperature and evaporated *in vacuo*. The residue was dissolved in water and extracted with ether–chloroform (1 : 1). The extract was washed with water, dried (Na_2SO_4), and evaporated, leaving a crystalline residue, which was separated by preparative t.l.c. to give crude [1-^{3}H]diosgenin (110 mCi). [1-^{3}H]*Diosgenin* (VI) was recrystallised from methanol to give needles (36 m Ci; 3 mg), identical with the authentic sample of diosgenin, m.p. 206—207° (mixed m.p., i.r. spectra, and t.l.c.).

[1-^{3}H]*Smilagenone* (X).—A solution of [1-^{3}H]diosgenone (VII) (1·55 × 10^9 decomp. min^{-1}) and carrier diosgenone (10 mg) in ethanol (4 ml) containing 20% sodium hydroxide (0·1 ml) was hydrogenated over 10% palladium–charcoal (10 mg). The mixture was filtered, evaporated *in vacuo*, and extracted with ethyl acetate. The extract was washed with water, dried (Na_2SO_4), and evaporated, leaving a crystalline residue, which was recrystallised from ethyl acetate to give [1-^{3}H]-*smilagenone* (X) (8 mg; 1·2 × 10^9 decomp. min^{-1}), identical with an authentic sample of smilagenone, m.p. 186—187° (mixed m.p., i.r. spectra, and t.l.c.).

[1-^{3}H]*Smilagenine* (VIII) *and* [1-^{3}H]*Epismilagenin* (IX).—A solution of (1-^{3}H]smilagenone (X) (5·5 × 10^8 decomp. min^{-1}; 4·5 mg) in tetrahydrofuran (3 ml) was added to a solution of lithium hydridotri-t-butoxyaluminate (30 mg) in tetrahydrofuran (10 ml) with stirring; the mixture was stirred for 2 h at room temperature, decomposed by addition of water (0·5 ml), and extracted with ethyl acetate. The extract was washed with water, dried (Na_2SO_4), and evaporated, leaving a crystalline residue (5·1 × 10^8 decomp. min^{-1}; 4·5 mg), which was separated by preparative t.l.c. on silica gel into [1-^{3}H]*smilagenin* (VIII) (0·4 mg; 5·0 × 10^7 decomp. min^{-1}) and [1-^{3}H]*epismilagenin* (IX) (3·5 mg; 4·1 × 10^8 decomp. min^{-1}). Compounds (VIII) and (IX) were shown to be identical with smilagenin, m.p. 184—185° (from methanol), and epismilagenin, m.p. 220—222° (from acetone), $[\alpha]_D^{27}$ −61·2° (*c* 0·237 in $CHCl_3$), respectively (mixed m.p. and t.l.c.).

General Procedure for Incubation of Labelled Compounds.—The plant seedlings (10—20 g), taken about 1 month after germination, were washed with sterilised water and homogenised with sterilised 0·067M-phosphate buffer (100 ml; pH 6·5) under nitrogen at 0—3° in an ice-bath for 1 min by use of a Nihon Seiki–Homogeniser (3000 rev. min^{-1}). A labelled compound * was incubated with the homogenate at 27—29° for 7 h in a Sakaguchi shaking flask fitted with a cotton stopper (shaking 110—120 times min^{-1}). Methanol (100 ml) was added and the mixture was heated under reflux for 5 h, then filtered. The residue was extracted three times with 80% methanol under reflux for 2 h. The combined methanolic solution was evaporated *in vacuo*. The product was hydrolysed with 5% sulphuric acid in methanol–water (2 : 1) and the resulting mixture extracted with chloroform. The extract was saponified with 5% potassium hydroxide in methanol and extracted with chloroform, and the latter extract was subjected to chromatography on alumina and preparative t.l.c. on silica gel. Fractions stated to be inactive were shown to be so by the recrystallisation method.

Incubation of [1-^{3}H]*diosgenin* (VI). The incubation mixture {from seedlings (21 g) and [1-^{3}H]diosgenin (6·0 × 10^8 decomp. min^{-1})} yielded a methanol extract of activity 4·8 × 10^8 decomp. min^{-1}. The chloroform extract (250 mg; 3·6 × 10^8 decomp. min^{-1}) was chromatographed on alumina to give the isodiotigenin fraction (*a*) (5·9 mg; 1·7 × 10^6 decomp. min^{-1}), the tokorogenin fraction (*b*) (4·0 mg; 1·7 × 10^6 decomp. min^{-1}), the yonogenin fraction (*c*) (3·0 mg; 8·2 × 10^6 decomp. min^{-1}), the monohydroxy-fraction (*d*) (4·0 mg; 2·8 × 10^8 decomp. min^{-1}), and the ketone fraction (*e*) (7·0 mg; 9·0 × 10^6 decomp. min^{-1}).

Fraction (*a*) was acetylated and separated to give isodiotigenin acetate (1·0 mg; 2·1 × 10^4 decomp. min^{-1}) by preparative t.l.c. This was recrystallised from methanol–acetone after addition of authentic isodiotigenin acetate (5·0 mg), giving radioinactive product. Fraction (*b*) was separated by preparative t.l.c. to give radioinactive tokorogenin (III). Fraction (*c*) was purified by preparative t.l.c. (three times) to give yonogenin (I) (0·5 mg; 4·4 × 10^6 decomp. min^{-1}), which was acetylated after addition of an authentic sample (5·0 mg). Preparative t.l.c. gave yonogenin acetate (5·0 mg; 1·3 × 10^6 decomp. min^{-1}), which was recrystallised from methanol after addition of an authentic sample (15 mg), giving radioactive yonogenin acetate (constant specific activity 1500 decomp. min^{-1} mg^{-1}; incorporation 0·0062%).

Fraction (*d*) was dissolved in benzene (1 ml) and pyridine (1 ml) and oxidised with osmium tetroxide (20 mg) at room temperature for 24 h. The mixture was decomposed with hydrogen sulphide and extracted with benzene. Authentic smilagenin (4 mg) was added to the extract; preparative t.l.c. gave smilagenin (3 mg; 9·59 × 10^6 decomp. min^{-1}). This was recrystallised from methanol after addition of authentic smilagenin (47 mg), giving radioactive smilagenin

* A labelled compound was dissolved in a solution of Tween-80 (0·3 ml) in methanol (1 ml) and added to a Sakaguchi shaking flask. Methanol was evaporated off *in vacuo* and then the homogenate was added. Additions of DPN, TPN, ATP, and G-6P as co-factors had no effect on incorporation.

(VIII) (constant specific activity $1\cdot1 \times 10^{4}$ decomp. min^{-1} mg^{-1}; incorporation 0·11%).

Fraction (*e*) was recrystallised from methanol after addition of authentic diosgenone (20 mg) to give radioactive diosgenone (VII) (constant specific activity $1\cdot5 \times 10^{5}$ decomp. min^{-1} mg^{-1}; incorporation 0·84%).

Incubation of [1-^{3}H]*diosgenone* (VII). The incubation mixture {from seedlings (15 g) and [1-^{3}H]diosgenone (VII) ($3\cdot6 \times 10^{8}$ decomp. min^{-1})} yielded a methanol extract of activity $1\cdot5 \times 10^{8}$ decomp. min^{-1}. The chloroform extract (130 mg; $1\cdot0 \times 10^{8}$ decomp. min^{-1}) was chromatographed on alumina to give fractions (*a*—*e*) as before: (*a*) (21 mg; $9\cdot1 \times 10^{5}$ decomp. min^{-1}), (*b*) (8 mg; $8\cdot6 \times 10^{5}$ decomp. min^{-1}), (*c*) (3 mg; $2\cdot0 \times 10^{6}$ decomp. min^{-1}), (*d*) (17 mg; $6\cdot6 \times 10^{7}$ decomp. min^{-1}), and (*e*) (10 mg; $2\cdot05 \times 10^{7}$ decomp. min^{-1}).

Fraction (*a*) was purified by preparative t.l.c. to give the product (17 mg; $1\cdot67 \times 10^{5}$ decomp. min^{-1}), which was acetylated and purified by preparative t.l.c. to give isodiotigenin acetate (7 mg; 6600 decomp. min^{-1}). This was recrystallised from methanol to give radioinactive isodiotigenin acetate. Fraction (*b*) was separated by preparative t.l.c. to give inactive tokorogenin (III). Fraction (*c*) was purified by preparative t.l.c. (three times) to give yonogenin (I) (0·5 mg; $3\cdot06 \times 10^{5}$ decomp. min^{-1}), which was acetylated after addition of authentic yonogenin (13 mg). Preparative t.l.c. gave [illegible]nogenin acetate (12 mg; $1\cdot08 \times 10^{4}$ decomp. min^{-1}). [illegible]s was recrystallised from methanol to give radioactive yonogenin acetate (constant specific activity 400 decomp. min^{-1} mg^{-1}; incorporation 0·0032%).

Fraction (*d*) (17 mg; $6\cdot6 \times 10^{7}$ decomp. min^{-1}) was separated by preparative t.l.c. to give monohydroxy-compounds (3 mg; $1\cdot53 \times 10^{7}$ decomp. min^{-1}), which were acetylated and purified by preparative t.l.c. to give the acetate mixture (*f*) (1 mg; $4\cdot7 \times 10^{5}$ decomp. min^{-1}). The mixture (*f*) ($1\cdot0 \times 10^{5}$ decomp. min^{-1}) was recrystallised from methanol after addition of authentic diosgenin acetate (20 mg), giving radioinactive diosgenin acetate. Another sample of the mixture (*f*) ($9\cdot0 \times 10^{4}$ decomp. min^{-1}) was recrystallised from methanol after addition of authentic smilagenin acetate (50 mg), giving radioactive smilagenin acetate (constant specific activity 330 decomp. min^{-1} mg^{-1}; incorporation 0·057%). A third sample ($4\cdot5 \times 10^{4}$ decomp. min^{-1}) was recrystallised from methanol after addition of authentic 3β-acetoxyspirost-4-en (10 mg), giving the radioactive acetate of (XIV) (constant specific activity 3700 decomp. min^{-1} mg^{-1}; incorporation 0·25%). A fourth sample ($8\cdot4 \times 10^{4}$ decomp. min^{-1}) was recrystallised from n-hexane–acetone after addition of authentic epismilagenin acetate (17 mg), giving radioactive epismilagenin acetate (constant specific activity 180 decomp. min^{-1} mg^{-1}; incorporation 0·002%).

Incubation of [1-^{3}H]*smilagenone* (X). Seedlings (12·5 g) and [1-^{3}H]smilagenone (X) (2 mg; $2\cdot2 \times 10^{8}$ decomp. min^{-1}) were used. The methanol extract had activity $1\cdot5 \times 10^{8}$ decomp. min^{-1}. The chloroform extract (130 mg; $1\cdot35 \times 10^{8}$ decomp. min^{-1}) was chromatographed on alumina and then purified by preparative t.l.c. to give fractions (*a*—*d*) as before: (*a*) (15 mg; $3\cdot6 \times 10^{4}$ decomp. min^{-1}), (*b*) (6 mg; $5\cdot1 \times 10^{4}$ decomp. min^{-1}), (*c*) (3 mg; $8\cdot2 \times 10^{4}$ decomp. min^{-1}), (*d*) (5 mg; $5\cdot2 \times 10^{6}$ decomp. min^{-1}).

Fraction (*a*) was acetylated to give the acetate (17 mg; $3\cdot6 \times 10^{4}$ decomp. min^{-1}), which was purified by preparative t.l.c. to give radioinactive isodiotigenin acetate (16 mg; $8\cdot1 \times 10^{3}$ decomp. min^{-1}). Fraction (*b*) was acetylated to give the acetate (7 mg; $4\cdot6 \times 10^{4}$ decomp. min^{-1}), which was purified by preparative t.l.c. to give inactive tokorogenin acetate (4 mg; $4\cdot1 \times 10^{3}$ decomp. min^{-1}). Fraction (*c*) was acetylated to give the acetate (2 mg; $8\cdot04 \times 10^{4}$ decomp. min^{-1}), which was purified by preparative t.l.c. to give yonogenin acetate (0·5 mg; $7\cdot4 \times 10^{3}$ decomp. min^{-1}). This was recrystallised from methanol after addition of authentic yonogenin acetate (10 mg) to give radioactive yonogenin acetate (constant specific activity 300 decomp. min^{-1} mg^{-1}; incorporation 0·002%).

Fraction (*d*) was dissolved in benzene (1 ml) and pyridine (1 ml) and oxidised with osmium tetroxide (20 mg) for 24 h at room temperature. The mixture was decomposed with hydrogen sulphide and extracted with benzene. The product was separated by preparative t.l.c. to give the epismilagenin fraction (*e*) (0·2 mg; $1\cdot55 \times 10^{5}$ decomp. min^{-1}), the smilagenin fraction (*f*) (0·2 mg; $1\cdot8 \times 10^{5}$ decomp. min^{-1}), and the polyhydroxy-fraction (radioinactive). Fraction (*e*) ($7\cdot7 \times 10^{4}$ decomp. min^{-1}) was recrystallised from n-hexane–acetone after addition of authentic epismilagenin (10 mg), giving radioactive epismilagenin (IX) (constant specific activity 4670 decomp. min^{-1} mg^{-1}; incorporation 0·06%). The remaining fraction (*e*) ($7\cdot7 \times 10^{4}$ decomp. min^{-1}) was recrystallised from methanol after addition of authentic smilagenin (20 mg), giving radioinactive smilagenin.

Fraction (*f*) was recrystallised from methanol after addition of authentic smilagenin (15 mg), giving radioactive smilagenin (VIII) (constant specific activity 4600 decomp. min^{-1} mg^{-1}; incorporation 0·046%).

Incubation of [1-^{3}H]*smilagenin* (VIII). Seedlings (9 g) and [1-^{3}H]smilagenin (VIII) (2 mg; 5×10^{7} decomp. min^{-1}) were used. The methanol extract had activity $3\cdot4 \times 10^{7}$ decomp. min^{-1}. The chloroform extract (110 mg; $3\cdot9 \times 10^{7}$ decomp. min^{-1}) was chromatographed on alumina and purified by preparative t.l.c. to give the isodiotigenin fraction (*a*) (6 mg; $2\cdot7 \times 10^{4}$ decomp. min^{-1}), the tokorogenin fraction (*b*) (2 mg; $5\cdot7 \times 10^{4}$ decomp. min^{-1}), the yonogenin fraction (*c*) (2 mg; $1\cdot4 \times 10^{5}$ decomp. min^{-1}), the monohydroxy-fraction (*d*) (2 mg; $2\cdot77 \times 10^{6}$ decomp. min^{-1}), the smilagenin fraction (*e*) (2 mg; $3\cdot24 \times 10^{7}$ decomp min^{-1}), and the ketone fraction (*f*) (0·5 mg; $2\cdot4 \times 10^{6}$ decomp min^{-1}).

Fraction (*a*) was acetylated and purified by preparative t.l.c. to give inactive isodiotigenin acetate (6 mg; 3300 decomp. min^{-1}). Fraction (*b*) gave inactive tokorogenin acetate (2 mg; 6600 decomp. min^{-1}). Fraction (*c*) gave yonogenin acetate (2 mg; $4\cdot7 \times 10^{4}$ decomp. min), which was recrystallised from methanol after addition of authentic yonogenin acetate (22 mg) to give radioactive yonogenin acetate (constant specific activity 200 decomp. min^{-1} mg^{-1}; incorporation 0·014%).

Fraction (*f*) was purified by preparative t.l.c. after addition of authentic smilagenone (5 mg) to give smilagenone (5 mg; $2\cdot8 \times 10^{5}$ decomp. min^{-1}). This was crystallised from methanol after addition of authentic smilagenone (10 mg), giving radioactive smilagenone (X) (constant specific activity $1\cdot8 \times 10^{4}$ decomp. min^{-1} mg^{-1}; incorporation 0·79%).

Incubation of [1-^{3}H]*epismilagenin* (IX). Seedlings (12·5 g) and [1-^{3}H]epismilagenin (IX) (2 mg; $2\cdot0 \times 10^{8}$ decomp. min^{-1}) were used. The methanol extract had activity $1\cdot5 \times 10^{8}$ decomp. min^{-1}. The chloroform extract (125 mg; $1\cdot6 \times 10^{8}$ decomp. min^{-1}) was chromatographed on alumina

and purified by preparative t.l.c. to give the isodiotigenin fraction (*a*) (16 mg; $3{\cdot}24 \times 10^4$ decomp. min^{-1}), the tokorogenin fraction (*b*) (6 mg; $3{\cdot}87 \times 10^4$ decomp. min^{-1}), the yonogenin fraction (*c*) (0·5 mg; $3{\cdot}6 \times 10^5$ decomp. min^{-1}), the monohydroxy-fraction (*d*) (3 mg; $6{\cdot}6 \times 10^7$ decomp. min^{-1}), and the ketone fraction (*e*) (0·5 mg; $2{\cdot}1 \times 10^6$ decomp. min^{-1}).

Fraction (*a*) was acetylated and purified by preparative t.l.c. to give isodiotigenin acetate (16 mg; 6000 decomp. min^{-1}). This was recrystallised from methanol after addition of authentic isodiotigenin acetate (10 mg), giving radioactive isodiotigenin acetate (constant specific activity 48 decomp. min^{-1} mg^{-1}; incorporation 0·00083%). Fraction (*b*) gave inactive tokorogenin acetate (5 mg; $1{\cdot}3 \times 10^4$ decomp. min^{-1}). Fraction (*c*) was acetylated after addition of authentic yonogenin (10 mg) and the product was purified by preparative t.l.c. to give yonogenin acetate (10 mg; $9{\cdot}96 \times 10^4$ decomp. min^{-1}). This was recrystallised from methanol after addition of authentic yonogenin acetate (10 mg), giving radioactive yonogenin acetate (constant specific activity 1350 decomp. min^{-1} mg^{-1}; incorporation 0·018%).

Fraction (*e*) was purified by preparative t.l.c. after addition of authentic smilagenone (10 mg) to give smilagenone (10 mg; $6{\cdot}9 \times 10^5$ decomp. min^{-1}). This was recrystallised from methanol after addition of authentic smilagenone (10 mg), giving radioactive smilagenone (X) (constant specific activity $3{\cdot}0 \times 10^4$ decomp. min^{-1} mg^{-1}; incorporation 0·4%).

[1/1476 *Received*, 16*th August*, 1971]