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STUDIES ON THE STEROIDAL COMPONENTS OF DOMESTIC PLANTS—XLI[1]

CONSTITUENTS OF *REINECKIA CARNEA* KUNTH (6)[2] STRUCTURE OF REINECKIAGENIN, ISOREINECKIAGENIN AND ISOCARNEAGENIN[3]

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Abstract—Convallamarogenin (Ia), isorhodeasapogenin (IIIa), isoreineckiagenin (VIIa), reineckiagenin (VIIIa) and isocarneagenin (XIIIa) were isolated from *Reineckia carnea* KUNTH. The three sapogenins, isoreineckiagenin, reineckiagenin and isocarneagenin are new and are the only known stereoidal sapogenins isolated from a plant source containing a hydroxyl group in the F-ring.

DURING recent years, the steroidal components of *Reineckia carnea* KUNTH have been investigated by our group and β-sitosteryl-*d*-glucoside,[4] β-sitosterol,[4] diosgenin,[5] kitigenin[5,6] and pentologenin[6] were isolated. The structures of kitigenin and pentologenin have recently been established.

As the paper chromatography of the methanol extract of the whole plant showed three spots having *Rf* values of 0·78, 0·58 and 0·55 in addition to those of the above-mentioned components (see Table 1), we now wish to report the results of the structural investigation of the sapogenins having these *Rf* values.

TABLE 1. PAPER CHROMATOGRAM OF EXTRACTS

Substance	*Rf* values		
	Benzene-extract	$CHCl_3$-extract	$CHCl_3$–CH_3OH extract
β-Sitosterol	0·94	—	—
Diosgenin	0·88	—	—
Compound A	0·78	0·78	—
Compound B	0·78	0·78	—
Compound C	0·58	0·58	—
Compound D	0·55	0·55	—
Compound E	0·55	0·55	—
Kitigenin	0·36	0·36	0·36
Pentologenin	0·11	0·11	0·11

[1] Part XL: T. Okanishi, A. Akahori and F. Yasuda, *Chem. Pharm. Bull., Japan.* **10,** 1195 (1962).
[2] (5): K. Sasaki, *Chem. Pharm. Bull., Japan* **9,** 693 (1961).
[3] A preceding communication: *Tetrahedron Letters.* No. 24, 1107 (1962).
[4] T. Okanishi and A. Shimaoka, *Ann. Rept. Shionogi Research Lab.* **10,** 1391 (1960).
[5] K. Takeda, T. Okanishi and A. Shimaoka, *Yakugaku Zasshi* **75,** 560 (1955).
[6] K. Takeda, T. Okanishi, K. Sasaki and A. Shimaoka, *Chem. Pharm. Bull., Japan* **9,** 631 (1961); K. Sasaki, *Ibid.* **9,** 684, 693 (1961).

From the neutral fraction of the saponified methanol extract of the dried whole plant (39 kg) we obtained the following fractions: benzene extract (100 g), chloroform extract (100 g) and chloroform–methanol (4:1) extract (300 g). The benzene extract afforded five sapogenins by alumina chromatography and recrystallization (see Table 2).

TABLE 2. SAPOGENINS OBTAINED FROM BENZENE EXTRACT (100 g)

	m.p.	$[\alpha]_D$	*Rf* value	Yield (g)
Compound A (Convallamarogenin, Ia)	259–61°	−79·1°	0·76	6·0
Compound B (Isorhodeasapogenin, IIIa)	238–40°	−76·3°	0·78	8·0
Compound C (Isoreineckiagenin, VIIa)	240–2°	−65·7°	0·58	0·06
Compound D (Reineckiagenin, VIIIa)	278–80°	−70·6°	0·55	0·03
Compound E (Isocarneagenin, XIIIa)	242–4°	−63·4°	0·55	0·015
(Carneagenin,* XIV)	262–4°	−71·6°	0·55	—

* Synthesized

Compound A (Ia), *Rf* value of 0·76, has the empirical formula $C_{27}H_{42}O_4$ and affords a diacetate (Ib), m.p. 214—215° on acetylation with acetic anhydride–pyridine at room temperature; thus Ia possesses two hydroxyl groups.

The I.R. spectrum of Ia shows three absorption bands at 3095, 1655 and 877 cm^{-1} corresponding to the $\rangle C{=}CH_2$ type double bond. Moreover, on catalytic hydrogenation of Ia in ethanol with 10% palladium charcoal, 1 mole of hydrogen is taken up smoothly and a reduction product corresponding to the dihydro derivative obtained. This compound no longer shows absorption bands corresponding to the vinyl type double bond in the I.R. spectrum, and affords two isomeric dihydro derivatives (II, m.p. 280–281° and IIIa, m.p. 238–240°). Since compound II and IIIa are identical in all respects (mixed m.p. determination and comparisons of I.R. spectra and $[\alpha]_D$ values) with rhodeasapogenin[7]* and isorhodeasapogenin[8]* respectively, it may be concluded that the double bond of Ia is situated at C-25(27) and that compound A (Ia) is $\Delta^{25(27)}$ 1β,3β-dihydroxy-5β-spirostane.

This structure was confirmed chemically as mentioned below.

As shown in Chart 1, Ib yields a dihydroxy derivative (IV, m.p. 213–214°) and a small amount of its isomer (V, m.p. 204–205°) on oxidation with osmium tetroxide. When IV is oxidized with lead tetracetate, it yields a carbonyl compound (VIb, m.p. 177–178°), the I.R. spectrum of which shows an absorption bond at 1729 cm^{-1}† corresponding to the six-membered ring ketone. On Wittig reaction of VIb with methyl-triphenyl-phosphonium bromide, Ia is obtained as expected.

From the results of catalytic hydrogenation and Wittig reaction, the constitution of compound A has been established as $\Delta^{25(27)}$-5β,20β,22α-spirostane-diol-(1β,3β),

* The authors are very much indebted to Dr. K. Morita (Takeda Research Lab.) for sending them the samples of rhodeasapogenin and isorhodeasapogenin.

† The I.R. spectrum of 20-keto-sapogenine showed a frequency at 1762 cm^{-1} (five-membered ring ketone). M. E. Wall and H. A. Walens, *Chem. & Ind.* 818 (1957).

[7] H. Nawa, *Yakugaku Zasshi* **73**, 1193 (1953).

[8] K. Morita, *Bull. Chem. Soc., Japan* **32**, 791, 794 (1959).

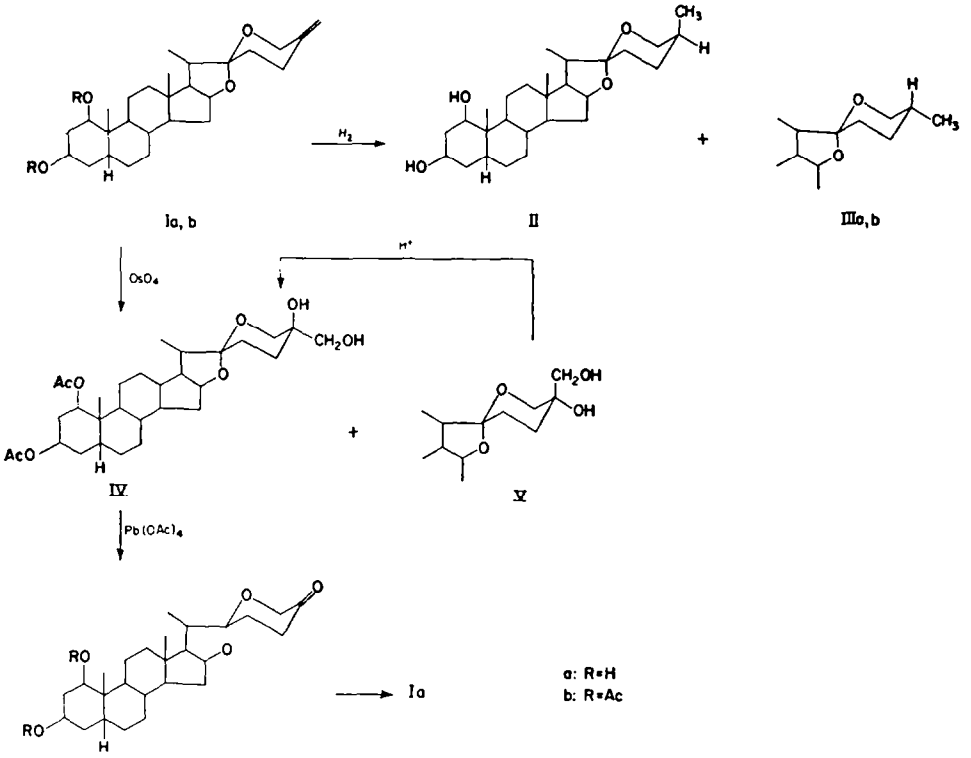


Chart I

Recently, Tschesche[9]* isolated a new sapogenin, convallamarogenin from *Convallaria majalis* L. and confirmed its structure to be Ia and convallamarogenin (Ia) was also isolated from *Reineckia carnea* KUNTH by our investigation.

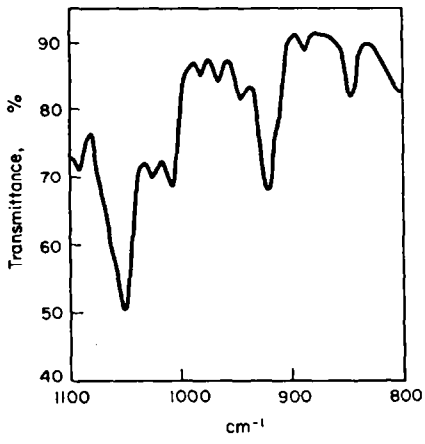
Compound B (IIIa) having *Rf* value of 0·78 was obtained from the mother liquid by recrystallization of convallamarogenin (Ia), and shown to be isorhodeasapogenin by mixed m.p. determination and comparisons of I.R. spectra and $[\alpha]_D$ values.

The new sapogenins, compound C and D, both corresponded to the empirical formula $C_{27}H_{44}O_5$. Compound C, isoreineckiagenin (VIIa) having *Rf* value of 0·58, affords a diacetate (VIIb), m.p. 202–204° on acetylation with acetic anhydride–pyridine at room temperature, but a triacetate (VIIc), m.p. 133–135° is obtained together with the diacetate (VIIb) on acetylation at 90–100°,

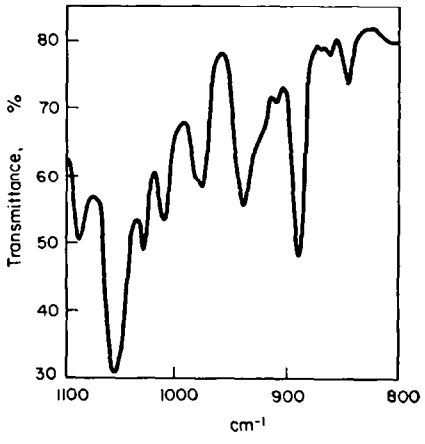
Under the same conditions, compound D, reineckiagenin (VIIIa) having *Rf* value of 0·55, yields a diacetate (VIIIb), m.p. 198–200° and a triacetate (VIIIc), m.p. 195–198°. Therefore, it is evident that isoreineckiagenin (VIIa) and reineckiagenin (VIIIa) possess three hydroxyl groups, and the third hydroxyl group may be a tertiary one.

* The authors are very grateful to Prof. R. Tschesche for sending them the samples of convallamarogenin and for his valuable discussion.

[9] R. Tschesche, H. Schwarz and G. Snatzke, *Chem. Ber.* **94**, 1699 (1961).

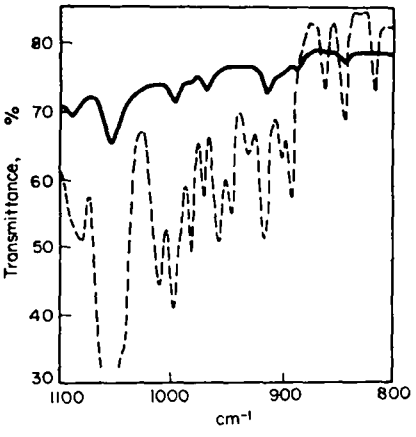


I.R. spectrum (in $CHCl_3$) of reineckiagenin (VIIIa)

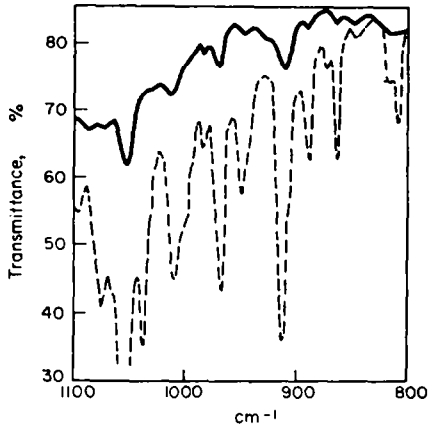


I.R. spectrum (in $CHCl_3$) of isoreineckiagenin (VIIa)

Fig. 1.



I.R. spectra of carneagenin (XIV)
—— : in $CHCl_3$
– – – : Nujol



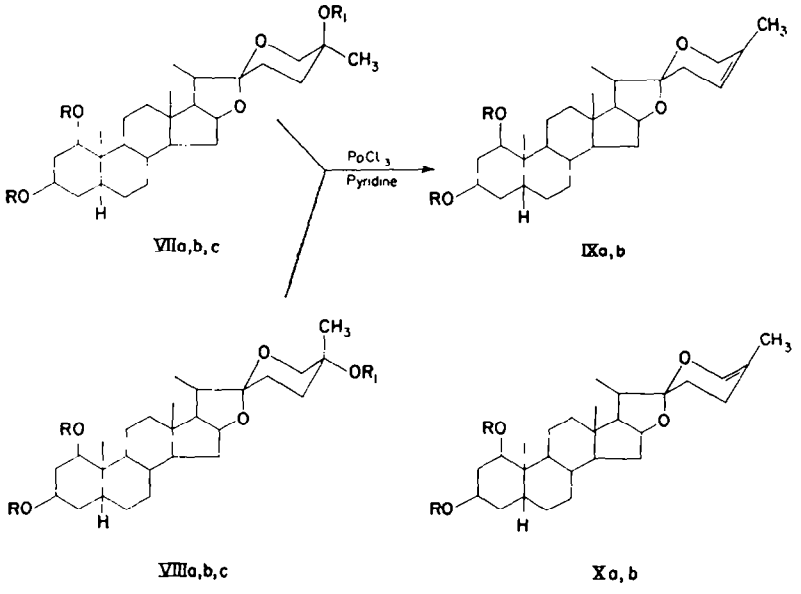
I.R. spectra of isocarneagenin (XIIIa)
—— : in $CHCl_3$
– – – : Nujol

Fig. 2.

The I.R. spectra of VIIa and VIIIa show that the characteristic bands[10] of the E- and F-rings have undergone a considerable change, but that they may belong to the 25D and 25L series[11] respectively (see Fig. 1).

When VIIb and VIIIb are dehydrated with phosphorus oxychloride in pyridine, both yield IXb, m.p. 178–181° and Xb, m.p. 108–112°/143–145° (see Chart 2). The N.M.R spectra of IXb and Xb show a signal at 8·42τ and 8·45τ respectively corresponding to a methyl group on a double bond; and one proton on the double bond in IXb gives rise to a signal centered at 4·60τ whereas that in Xb appears as a signal at 3·94τ, corresponding to a proton attached to a carbon atom attached to an oxygen atom.

[10] R. N. Jones, *J. Amer. Chem. Soc.* **75**, 158 (1953).
[11] M. E. Wall, C. R. Eddy, M. L. Meclenman and M. E. Klump, *Analyt. Chem.* **24**, 1337 (1952).



a: R, R_1 = H
b: R = Ac, R_1 = H
c: R, R_1 = Ac

Chart 2

From these results, it may be concluded that the tertiary hydroxyl groups of VIIa and VIIIa can only be situated at C-25. In order to confirm the structures of VIIa and VIIIa, these compounds were synthesized from convallamarogenin (Ia).

As shown in Chart 3, reaction of the carbonyl compound (VIb) with methylmagnesium iodide yields reineckiagenin (VIIIa) and a small amount of isoreineckiagenin (VIIa), as expected. Moreover, oxidation of convallamarogenin (Ia) with monoperphthalic acid gives two isomeric epoxides, XI, m.p. 230–232° and XII, m.p. 282–283°. On treatment of the two isomeric epoxides with lithium aluminum hydride, VIIa is obtained from XI and VIIIa from XII. Reduction of the monotosylate of the dihydroxy derivative (IV) of convallamarogenin diacetate (Ib) with lithium aluminum hydride again yields isoreineckiagenin (VIIa). From these results, the constitutions of isoreineckiagenin (VIIa) and reineckiagenin (VIIIa) have been established as 5β-spirostane-1β,3β,25-triols.

As to the configuration of the C-25 hydroxyl group, the I.R. spectrum of isoreineckiagenin diacetate (VIIb) shows an absorption band at 3594 cm^{-1} corresponding to the intramolecular hydrogen bond, whereas that of reineckiagenin diacetate (VIIIb) shows an absorption band at 3610 cm^{-1} corresponding to the free hydroxyl group.[12] These facts support the assumption that VIIa and VIIIa belong to 25D and 25L series respectively.

The N.M.R spectrum of isoreineckiagenin (VIIa) shows a signal at 64 c.p.s. (8.93τ) and that of reineckiagenin (VIIIa) shows a signal at 76 c.p.s. (8.73τ) referred to tetramethylsilane, corresponding to the C-27 methyl group. The upward shift of

[12] M. Mousseron-Canet, M. Mousseron and C. Levallois, *C.R. Acad Sci., Paris* **253**, 1386 (1961).

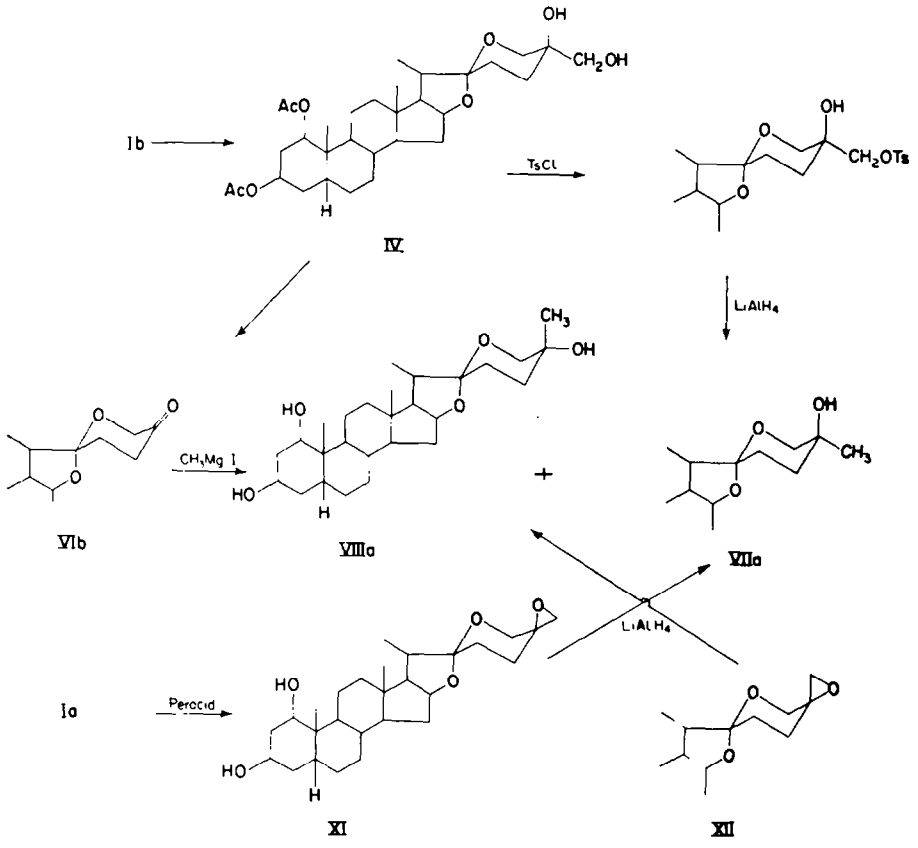


Chart 3

the signal from 76 to 64 c.p.s. has been attributed to the change of the C-27 methyl group from axial to equatorial.[13]

Therefore, the C-27 methyl groups in isoreineckiagenin (VIIa) and reineckiagenin (VIIIa) possess the equatorial (25D) and the axial configuration (25L), respectively. Since isoreineckiagenin (VIIa) is also obtained from the compound IV as mentioned above, the C-27 methylol group of IV should possess the equatorial configuration (25D) and its isomer (V) should be represented by the 25L formula.

Compound E, isocarneagenin (XIIIa, $C_{27}H_{44}O_5$) having *Rf* value of 0.55 affords a triacetate (XIIIb), m.p. 215–218° on acetylation with acetic anhydride–pyridine at room temperature.

Since the I.R. spectrum of XIIIa also shows that the characteristic bands of the E- and F-rings have undergone a marked change (see Fig. 2) and XIIIa is obtained together with convallamarogenin (Ia), isoreineckiagenin (VIIa) and reineckiagenin (VIIIa), it may be reasonable to suppose that the hydroxyl group of isocarneagenin (XIIIa) is situated at C-27.

Treatment of Ia with diborane yields two isomeric triols, m.p. 242–244° (XIIIa) and 262–264° (XIV) as shown in Chart 4. The former is identical with isocarneagenin

[13] W. E. Rosen, J. B. Ziegler, A. C. Shabica and J. N. Shoolery, *J. Amer. Chem. Soc.* **81**, 1687 (1959).

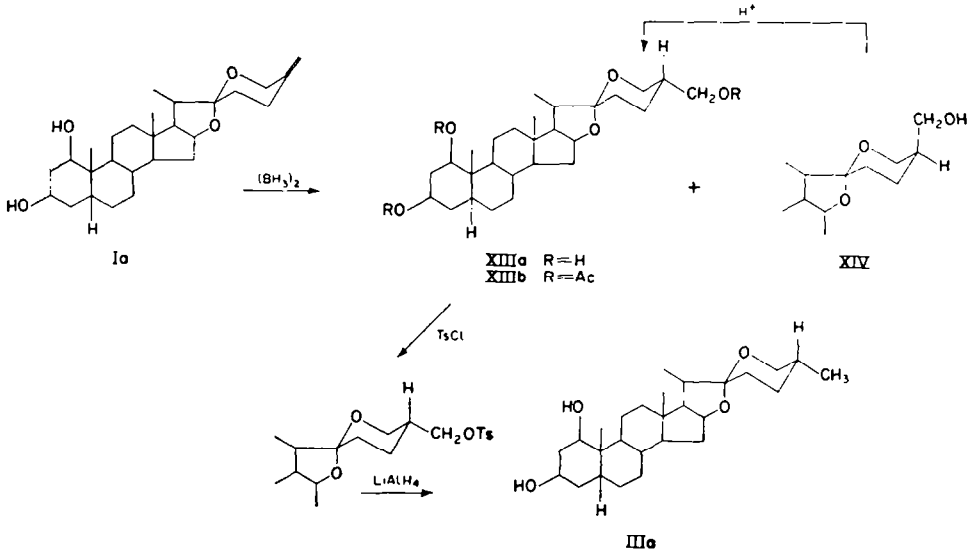


Chart 4

by mixed m.p. determination and comparison of I.R. spectra. Reduction of the monotosylate of isocarneagenin (XIIIa) with lithium aluminum hydride yields isorhodeasapogenin (IIIa), thus the C-27 methylol group of XIIIa should possess the equatorial configuration (25D) and its isomer (XIV) should be represented by the 25L formula. We wish to give the name "Carneagenin" to this isomer (XIV).

Carneagenin (XIV) is isomerized almost quantitatively to isocarneagenin (XIIIa) under extremely mild conditions as compared with the conditions required for isomerization of 25L to 25D sapogenin, e.g. it only requires 4% ethanolic hydrochloric acid at room temperature for one hour. Moreover, under similar conditions, compound V is also isomerized to IV. These facts outlined in Chart 5 show that

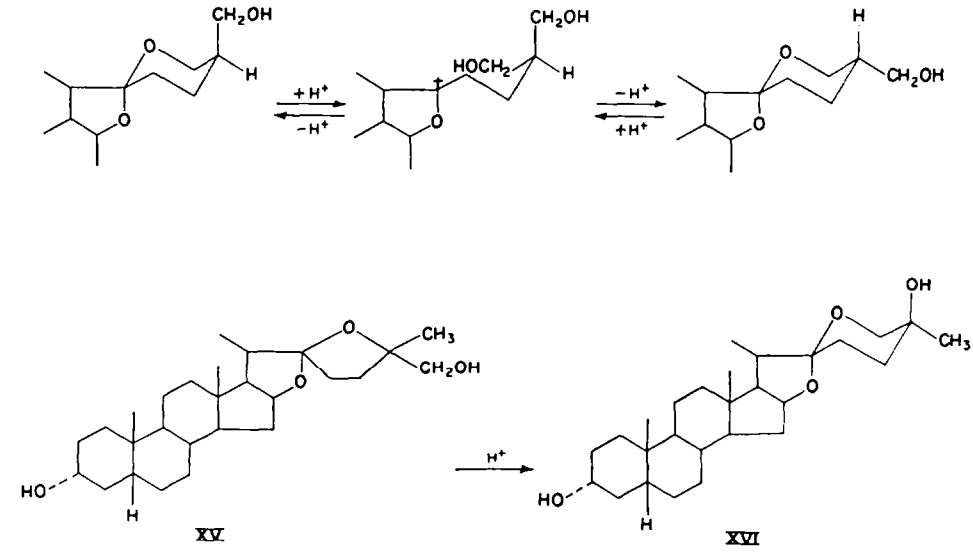


Chart 5

isocarneagenin (XIIIa) and compound IV are the more stable isomers. Assuming that isomerization of carneagenin to isocarneagenin takes place on saponification of the saponins, the question remains as to whether isocarneagenin is the naturally occurring sapogenin.

The Mosettig group[14] reported on the structures of cholegenin and isocholegenin,[15] isolated from ox bile, and established their structures as XV and XVI. They noted that cholegenin (XV) having a five-membered F-ring, was easily isomerized to isocholegenin (XVI) by dilute ethanolic hydrochloric acid at room temperature (see Chart 5).

This result, raises some doubts as to whether isoreineckiagenin (VIIa) or reineckiagenin (VIIIa) exist in the plant body as a proto-type glycoside having a five-membered F-ring.

However, it is noteworthy that isoreineckiagenin, reineckiagenin and isocarneagenin are the only known steroidal sapogenins isolated from a plant source containing a hydroxyl group in the F-ring, and that isocarneagenin possesses a primary alcohol function at C-27.

EXPERIMENTAL

N.M.R spectra were taken on deuterated chloroform solutions with a Varian A-60 N.M.R. Spectrometer. All m.p. were measured on a Kofler block ("mono-scope" Hans Bock Co. Ltd., Frankfurt am Main, Germany) and uncorrected. Paper partition chromatography was carried out with toluene-acetic acid (50:3) by the ascending method; detection reagent,[16] 1% solution of cinnamic aldehyde in ethanol and a solution of antimony trichloride (25 g) in nitrobenzene (5 ml).

Isolation of the sapogenins from the whole plant

The dried and sliced whole plant (39 kg) was extracted with 90% hot methanol giving a deep brown syrup (13·85 kg). The extract was dissolved in a solution of H_2SO_4 (1 kg) in 70% ethanol (20 l.), refluxed for 6 hr in a steam bath and poured into sufficient water to separate the crude sapogenins from this solution. The mixture was filtered and the insoluble sapogenins extracted with methanol giving a dark brown syrup (1·7 kg). The latter was refluxed with 4% KOH–methanol (6 l.) for 2 hr in a steam bath, poured into a large volume of water and extracted with benzene (5 l. × 3), chloroform (5 l. × 3) and then chloroform–methanol (4:1, 5 l. × 3). The benzene (100 g), chloroform (100 g) and chloroform–methanol (4:1) extract (300 g) gave the paper chromatograms shown in Table 1.

Isolation of the compounds A, B, C, D and E *from the benzene extract*

The benzene extract (100 g) was dissolved in benzene (1 l.) and chromatographed on alumina (1 kg) as shown in Table 3.

(1) *Compound* A (*convallamarogenin*, Ia). Fractions 30–38 were crystallized from chloroform–methanol (1:1) giving crude compound A (6·0 g), m.p. 250–257°. Recrystallization from the same solvent 4 times gave compound A, convallamarogenin (Ia) as colourless prisms, m.p. 259–261°, $[\alpha]_D^{27}$ −79·1° (±2°) (*c* 1·018, chloroform), ν_{max}^{Nujol} 3350, 3295, 3095,1655 and 877 cm^{-1}, *Rf* value 0·76 (Found: C, 75·05; H, 9·93. $C_{27}H_{42}O_4$ requires: C, 75·31; H, 9·83%). Diacetate (Ib), colourless prisms, m.p. 214–215°, $[\alpha]_D^{27}$ −88·2° (+2°) (*c* 1·097, chloroform) (Found: C, 72·23; H, 9·11. $C_{31}H_{46}O_6$ requires: C, 72·34; H, 9·01%).

(2) *Compound* B (*isorhodeasapogenin*, IIIa). The mother liquor after recrystallization of compound A was evaporated leaving a crystalline residue, which was fractionally recrystallized from methanol giving crude compound B (8 g), m.p. 220–232° and a mixture of compounds A and B, m.p. 220–245°. Recrystallization of the crude B from methanol, five times yielded B (IIIa) as colourless needles, m.p.

[14] M. J. Thompson, I. Scheer and E. Mosettig, *J. Amer. Chem. Soc.* **81**, 5225, 5222 (1959).

[15] W. H. Pearlman, J. *Amer. Chem. Soc.* **66**, 806 (1944); *J. Biol. Chem.* **166**, 473 (1946); W. H. Pearlman and E. Cerceo, *Ibid.* **176**, 847 (1948). N. J. Antia, Y. Mazur, R. R. Wilson and F. S. Spring, *J. Chem. Soc.* 1218 (1954); Y. Mazur and F. S. Spring, *Ibid.* 1223 (1954).

[16] T. Okanishi, A. Akahori and F. Yasuda, *Ann. Rept. Shionogi Research Lab.* **8**, 927 (1958).

TABLE 3. ALUMINA CHROMATOGRAM OF THE BENZENE EXTRACT

Fraction no.	Solvent	*Rf* value	Yield (g)
1–4	Benzene	0·94	29·5
5–12	Benzene–chloroform (9:1)	0·94	11·0
13–22	Benzene–chloroform (4:1)	0·88	6·0
23–29	Benzene–chloroform (1:1)	0·88	12·0
30–38	Chloroform	0·78	25·0
39–42	Chloroform–methanol (95:5)	0·78, 0·58, 0·55	5·0
43–44	Chloroform–methanol (95:5)	0·55, 0·36	1·0
45–50	Chloroform–methanol (4:1)	0·55, 0·36	3·0
50–56	Chloroform–methanol (1:1)	0·36, 0·11	1·0

239–240°, $[\alpha]_D^{26}$ −76·3° (±3°) (*c* 0·692, chloroform), *Rf* value 0·78 (Found: C, 74·92; H, 10·20. $C_{27}H_{44}O_4$ requires: C, 74·95; H, 10·25%). Diacetate (IIIb), colourless prisms, m.p. 203–205°, $[\alpha]_D^{28}$ −66·5° (±4°) (*c* 0·647, chloroform) (Found: C, 72·23; H, 9·34. $C_{31}H_{48}O_6$ requires: C, 72·06; H, 9·36%). Compound B is identical with isorhodeasapogenin (IIIa) by mixed m.p. determination and comparisons of I.R. spectra and $[\alpha]_D$ values.

(3) *Compounds* C, D *and* E. Fractions 39–42 (5 g) were dissolved in benzene (300 ml) and rechromatographed on alumina (250 g) as shown in Table 4.

TABLE 4. ALUMINA CHROMATOGRAM OF FRACTIONS 39–42

Fraction no.	Solvent	*Rf* value	Yield (mg)
1– 3	Benzene	0·98	41
4– 6	Benzene–chloroform (8:2)	0·98	34
7–23	Benzene–chloroform (7:3)	0·93, 0·77	620
24–27	Benzene–chloroform (6:4)	0·77, 0·58	195
28–29	Benzene–chloroform (6:4)	0·58	80
30–35	Benzene–chloroform (5:5)	0·58, 0·55	300
36–42	Benzene–chloroform (4:6)	0·55	210
43–46	Benzene–chloroform (3:7)	0·55	176
47–55	Benzene–chloroform (2:8)	0·55	410
56–63	Chloroform	0·55	300
64–78	Chloroform–methanol (98:2∼9:1)	0·55, 0·36	1930

(a) *Compound* C (*isoreineckiagenin*, VIIa). Fractions 28–35 were crystallized from ethyl acetate yielding crude C (60 mg), m.p. 238–242°. Recrystallization from ethyl actetate–petroleum ether gave C, isoreineckiagenin (VIIa) as colourless plates, m.p. 240–242°, $[\alpha]_D^{23}$ −65·7° (±5°) (*c* 0·376, dioxane), N.M.R 8·93τ (C-27 CH_3), *Rf* value 0·58 (Found: 72·00; H, 10·00.C_{27} $H_{44}O_5$ requires: C, 72·28; H, 9·89%). Diacetate (VIIb), colourless needles, m.p. 202—204°, $[\alpha]_D^{24}$ −87·0° (±4°) (*c* 0·431, chloroform), $\nu_{max}^{CCl_4}$ 3594 cm^{-1} (Found: C, 70·04; H, 9·30. $C_{31}H_{48}O_7$ requires: C, 69·89; H, 9·08%). Triacetate (VIIc), colourless prisms, m.p. 133–135°, $[\alpha]_D^{24}$ −74° (±5°) (*c* 0·292, chloroform) (Found: C, 69·12; H, 8·80. $C_{38}H_{50}O_8$ requires: C, 68·96; H, 8·77%).

(b) *Compound* D (*reineckiagenin*, VIIIa). Fractions 43–46 were crystallized from chloroform–methanol giving crude D (30 mg), m.p. 272–280°. Recrystallization from the same solvent gave D, reineckiagenin (VIIIa) as colourless prisms, m.p. 278–280°, $[\alpha]_D^{23}$ −70·6° (±4°) (*c*, 0·469, dioxane), N.M.R 8·73τ (C-27 CH_3), *Rf* value 0·55 (Found: C, 72·26; H, 10·10. $C_{27}H_{44}O_5$ requires: C, 72·28; H, 9·89%). Diacetate (VIIIb), colourless needles, m.p. 198–200°, $[\alpha]_D^{24}$ −82·0° (±5°) (*c*, 0·295, chloroform), $\nu_{max}^{CCl_4}$ 3610 cm^{-1} (Found: C, 69·72; H, 8·87. $C_{31}H_{48}O_7$ requires: C, 69·89; H, 9·08%). Triacetate (VIIIc), colourless plates, m.p. 195–198°, $[\alpha]_D^{24}$ −68·8° (±5°) (*c*, 0·353, chloroform) (Found: C, 69·20; H, 9·08. $C_{38}H_{50}O_8$ requires: C, 68·96; H, 8·77%).

(c) *Compound* E (*isocarneagenin*, XIIIa). Fractions 56–63 were crystallized from ethyl acetate giving crude E (15 mg), m.p. 236–240°. Recrystallization from the same solvent gave E, isocarneagenin (XIIIa) as colourless needles, m.p. 242–244°, $[\alpha]_D^{25}$ −63·4° (±5°) (*c* 0·286, methanol–chloroform (1:1)), *Rf* value 0·55 (Found: C, 72·27; H, 9·68. $C_{27}H_{44}O_5$ requires: C, 72·28; H, 9·89%). Triacetate (XIIIb), colourless needles, m.p. 215–218°, $[\alpha]_D^{24}$ −75·5° (±5°) (*c*, 0·273, chloroform) (Found: C, 69·08; H, 8·88. $C_{33}H_{50}O_8$ requires: C, 68·96; H, 8·77%).

Catalytic hydrogenation of convallamarogenin (Ia)

A mixture of 10% palladized charcoal (100 mg) and Ia (250 mg) in ethanol (50 ml) was reduced catalytically at room temp and atm. press. giving a crystalline product (240 mg). Recrystallization from ethanol gave colourless needles A (110 mg), m.p. 255–260° and colourless needles B (120 mg), m.p. 238–240°. The crystalline substance A was recrystallized from chloroform-methanol giving colourless needles (15 mg), m.p. 280–281°, $[\alpha]_D^{29}$ −31·6° (±2°) (*c*, 1·007, chloroform) (Found: C, 75·05; H, 9·93. $C_{27}H_{44}O_4$ requires: C, 74·95; H, 10·25%), and was shown to be rhodeasapogenin (II) by mixed m.p. and comparisons of I.R. spectra and $[\alpha]_D$ values. The crystalline substance B was acetylated with acetic anhydride–pyridine at 100° for 1 hr giving colourless prisms (64 mg,) m.p. 198–200°. Recrystallization from methanol gave colourless prisms, m.p. 203–205°, $[\alpha]_D^{28}$ −66·4° (±4°) (*c*, 0·647, chloroform) (Found: C, 72·23; H, 9·34. $C_{31}H_{48}O_6$ requires: C, 72·06; H, 9·36%), and was shown to be isorhodeasapogenin diaceate (IIIb) by mixed m.p. and I.R. comparison. Saponification of this acetate gave colourless needles, m.p. 239–240° (from ethanol), $[\alpha]_D^{26}$ −76·3° (±3°) (*c*, 0·692, chloroform) (Found: C, 74·92; H, 10·20. $C_{27}H_{44}O_4$ requires: C, 74·95; H, 10·25%), and was shown to be isorhodeasapogenin (IIIa) by mixed m.p. and comparisons of I.R. spectra and $[\alpha]_D$ values.

Osmium tetroxide oxidation of convallamarogenin diacetate (Ib)

A solution of osmium tetroxide (2 g) in dry pyridine (10 ml) was added to a solution of Ib (2 g) in the same solvent (10 ml) and left for 5 days at room temp. Dioxane (300 ml) was added to this mixture and saturated with hydrogen sulphide. The black precipitate was filtered off and well washed with dioxane. The filtrate and washings were evaporated *in vacuo*, extracted with chloroform, washed with 2N H_2SO_4, water and 2N Na_2CO_3, dried (Na_2SO_4) and evaporated leaving a syrup (2·39 g). The residue was dissolved in benzene (300 ml) and chromatographed on neutral alumina (90 g). Elution with benzene–chloroform (1:1) and chloroform afforded colourless needles (V, 40 mg), m.p. 204–205° (from acetone), $[\alpha]_D^{28}$ −58·4° (±2°) (*c*, 0·993, chloroform), *Rf* value 0·38 (Found: C, 68·05; H, 8·81. $C_{31}H_{48}O_8$ requires: C, 67·85; H, 8·82%). Further elution with chloroform and chloroform–methanol (9:1 and 8:2) afforded colourless prisms (IV, 780 mg), m.p. 213–214° (from methanol), $[\alpha]_D^{23}$ −84·0° (±2°) (*c*, 1·044, chloroform), *Rf* value 0·28 (Found: C, 67·67; H, 8·86. $C_{31}H_{48}O_8$ requires: C, 67·85; H, 8·82%).

Lead tetracetate oxidation of IV

A solution of IV (100 mg) in chloroform (2 ml) was added to a solution of 98·5% lead tetracetate (100 mg, 1·2 equivs.) in acetic acid (8 ml) and left for 10 hr at room temp. Water (50 ml) was added to this mixture, extracted with chloroform, washed with 5% $Na_2S_2O_3$, water and 2N Na_2CO_3, dried (Na_2SO_4) and evaporated *in vacuo* leaving a syrup (83 mg). The residue was dissolved in benzene (10 ml), filtered through a column of neutral alumina (3 g) and recrystallized from methanol giving colourless prisms (VIb, 66 mg), m.p. 177–178°, $[\alpha]_D^{25}$ −106·4° (±2°) (*c*, 1·062, chloroform), ν_{max}^{Nujol} 1727 cm^{-1} (C=O, OAc) (Found: C, 69·46; H, 8·58. $C_{30}H_{44}O_7$ requires: C, 69·74; H, 8·58%). VIb (53 mg) was saponified with 5% K_2CO_3–methanol giving colourless needles (VIa, 38 mg), m.p. 254–255°, $\nu_{max}^{chloroform}$ 3560 and 1729 cm^{-1} (C=O), *Rf* value 0·63 (Found: C, 70·79; H, 9·52. $C_{28}H_{40}O_5 \cdot \frac{1}{2}H_2O$ requires: C, 70·71; H, 9·28%).

Wittig reaction of VIb

Butyllithium—ether solution (0·95N, 2 ml) was added dropwise to a solution of methyltriphenylphosphonium bromide (750 mg) in dry ether (20 ml) with stirring at 0–5° in nitrogen atmosphere and stirred for an additional 1·5 hr in an ice bath. A solution of VIb (150 mg) in dry ether (25 ml) was then added dropwise to this mixture with stirring during 40 min, and additional stirring for 3 hr at

room temp and then evaporated to dryness. Dry tetrahydrofuran (30 ml) was added to the residue and refluxed for 2 hr on a steam bath. Water (60 ml) was added to the mixture, extracted with ether, washed with 2% HCl, water, saturated potassium bicarbonate, dried (Na_2SO_4) and evaporated leaving a syrup (150 mg). The residue was crystallized from methanol giving a crystalline substance (55 mg), m.p. 240–248°. The mother liquor after crystallization was evaporated and saponified with 5% K_2CO_3–methanol giving a crystalline substance (84 mg). These crystalline fractions were together and recrystallized from methanol–chloroform giving colourless prisms (92 mg), m.p. 258–261°, $[\alpha]_D^{26}$ −78·5° (±2°) (*c*, 0·980, chloroform) (Found; C, 75·11; H, 9·81. $C_{27}H_{42}O_4$ requires: C, 75·31; H, 9·83%), which was shown to be convallamarogenin (Ia) by mixed m.p. and comparisons of I.R. spectra and $[\alpha]_D$ values. Diacetate, colourless prisms, m.p. 214–215°, $[\alpha]_D^{25}$ −87·9° (±2°) (*c*, 1·013, chloroform) (Found: C, 71·99; H, 9·02. $C_{31}H_{46}O_6$ requires: C, 72·34; H, 9·01%) was also identical with convallamarogenin diacetate (Ib).

Dehydration of isoreineckiagenin diacetate (VIIb) *with phosphorus oxychloride–pyridine*

$POCl_3$ (100 mg) was added to a solution of VIIb (47 mg) in pyridine (1 ml) in an ice bath and left for 30 min at room temp. The mixture was heated at 60–70° for 2 hr, poured onto ice water, extracted with chloroform, washed with 2N H_2SO_4, water and 2N Na_2CO_3, dried (Na_2SO_4) and evaporated leaving a syrup (44 mg). The residue was dissolved in petroleum ether–benzene (4:1, 5 ml) and chromatographed on neutral alumina (4 g). Elution with petroleum ether-benzene (1:1) and benzene afforded colourless needles (Xb, 10 mg), m.p. 108–112°/143–145° (double m.p.) (from methanol), $[\alpha]_D^{24}$ −89·2° (±5°) (*c*, 0·342, chloroform), N.M.R 3·94 and 8·45τ (Found: C, 72·27; H, 8·98. $C_{31}H_{46}O_6$ requires: C, 72·34; H, 9·01%). Xa, colourless plates, m.p. 218–220° (from methanol), $[\alpha]_D^{25}$ −87·5° (±3°) (*c*, 0·854, chloroform) (Found: C, 75·12; H, 10·02. $C_{27}H_{42}O_4$ requires: C, 75·31; H, 9·83%). Further elution with benzene afforded colourless needles (IXb, 24 mg), m.p. 178–181° (from methanol), $[\alpha]_D^{24}$ −97·6° (±5°) (*c*, 0·211, chloroform), NMR 4·60 and 8·42τ (Found: C, 72·01; H, 9·12. $C_{31}H_{46}O_6$ requires: C, 72·34; H, 9·01%). IXa, colourless prisms, m.p. 282–284° (from methanol–chloroform), $[\alpha]_D^{25}$ −89·2° (±4°) (*c*, 0·408, chloroform) (Found: C, 74·93; H, 9·89. $C_{27}H_{42}O_4$ requires: C, 75·31; H, 9·83%).

Dehydration of reineckiagenin diacetate (VIIIb) *with phosphorus oxychloride–pyridine*

VIIIb (47 mg) was worked up as described above and chromatographed on neutral alumina. Xb (4 mg) and IXb (18 mg) were obtained, and the starting material (VIIIb, 7 mg) was recovered.

Grignard reaction of VIb *with methylmagnesium iodide*

A solution of VIb (300 mg) in dry ether (15 ml) and dry tetrahydrofuran (6 ml) was added dropwise to Grignard reagent, which was prepared from magnesium (185 mg) and methyl iodide (740 mg) in dry ether (40 ml), with stirring for 30 min at room temp and refluxed for 1·5 hr. NH_4Cl solution was then added to the mixture in an ice bath, extracted with chloroform. washed with water, dried (Na_2SO_4) and evaporated leaving a syrup (305 mg). The residue was recrystallized from methanol–chloroform giving colourless prisms (130 mg), m.p. 280–282°, $[\alpha]_D^{24}$ −76·2° (±4°) (*c*, 0·564, dioxane) (Found: C, 72·23; H, 10·09. $C_{27}H_{44}O_5$ requires: C, 72·28; H, 9·89%), which was identical with reineckiagenin (VIIIa). Alumina chromatography of the mother liquor after recrystallization gave VIIIa (55 mg) and colourless needles (3·5 mg), m.p. 230–235°, which was shown to be VIIa by mixed m.p. and I.R. comparison.

Peracid oxidation of convallamarogenin (Ia)

To a solution of Ia (500 mg) in chloroform (22 ml) a 4·8% solution of monoperphthalic acid in ether (5 ml, 1·2 equiv.) was added and left for 20 hr at room temp. Chloroform (100 ml) was then added and the mixture washed with 2% potassium iodide (30 ml), water, 1% sodium thiosulphate and water, dried (Na_2SO_4) and evaporated leaving a syrup (530 mg). The residue was crystallized from chloroform giving colourless needles A (100 mg), m.p. 270–275°. The mother liquor after crystallization was evaporated, and the residue was crystallized from acetone-methanol giving colourless needles B (53 mg), m.p. 210–215° and a mixture (300 mg) of crystalline compounds A and B. The crystalline A was recrystallized from methanol–chloroform giving colourless needles (XII, 42 mg), m.p. 282–283°, $[\alpha]_D^{24}$ −53·3° (±5°) (*c*, 0·276, pyridine) (Found: C, 72·29; H, 9·40. $C_{27}H_{42}O_5$ requires: C, 72·61;

H, 9·48%). Diacetate, colourless needles, m.p. 185–188° (Found: C, 70·05; H, 9·09. $C_{31}H_{46}O_7$ requires: C, 70·16; H, 8·74%). The crystalline B was recrystallized from acetone-methanol giving colourless needles (XI, 13 mg), m.p. 230–232°, $[\alpha]_D^{26}$ −68·7° (±5°) (*c*, 0·371, dioxane) (Found: C, 72·62; H, 9·56. $C_{27}H_{42}O_5$ requires: C, 72·61; H, 9·48%). Diacetate, colourless prisms, m.p. 228–231°, $[\alpha]_D^{21}$ −85·0° (±2°) (*c*, 1·060, chloroform) (Found: C, 70·29; H, 8·90. $C_{31}H_{46}O_7$ requires: C, 70·16; H, 8·74%).

Reduction of two isomeric epoxides with lithium aluminum hydride

(1) *Reduction of* XII. A solution of XII (30 mg) in dry tetrahydrofuran (6 ml) was added to a suspension of lithium aluminum hydride (50 mg) in dry tetrahydrofuran (1 ml) and stirred for 3 hr at room temp. The mixture was heated at 60° for 1hr, then decomposed by addition of water, extracted with chloroform, washed with water, dried (Na_2SO_4) and evaporated leaving a crystalline substance (30 mg). The residue was recrystallized from ethanol giving colourless prisms (27 mg), m.p. 280–282°, which was identical with reineckiagenin (VIIIa) by mixed m.p. and I.R. comparison.

(2) *Reduction of* XI. XI (30 mg) was reduced with lithium aluminum hydride as described above affording colourless needles (28 mg), m.p. 240–242°, $[\alpha]_D^{24}$ −61·4° (±4°) (*c*, 0·422, dioxane), which was identical with isoreineckiagenin (VIIa) by mixed m.p. and I.R. comparison.

(3) *Reduction of a mixture of* XI *and* XII. A mixture of XI and XII (250 mg) was reduced as described above and chromatographed on neutral alumina affording reineckiagenin (VIIIa, 38 mg) and isoreineckiagenin (VIIa, 82 mg).

Convertion of IV *into isoreineckiagenin* (VIIa)

Tosyl chloride (50 mg) was added to a solution of IV (50 mg) in pyridine (1 ml) and left 40 hr at room temp. A solution of the crude tosylate obtained (66 mg) in dry tetrahydrofuran (4 ml) was added to a suspension of lithium aluminum hydride (50 mg) in dry tetrahydrofuran (1 ml), refluxed with stirring for 3 hr, decomposed by addition of water and 2N H_2SO_4, extracted with chloroform, washed with water, 2N Na_2CO_3 and water, dried (Na_2SO_4) and evaporated leaving a crystalline substance (37 mg). The residue was recrystallized from methanol giving colourless needles (30 mg), m.p. 240–242°, identical with isoreineckiagenin (VIIa).

Hydroboration of convallamarogenin (Ia)

A solution of $BF_3{\cdot}Et_2O$ (270 mg) in dry diglyme (2 ml) was added dropwise to a solution of Ia (220 mg) and sodium borohydride (70 mg) in dry diglyme (7 ml) and dry tetrahedrofuran (18 ml) with stirring in an ice bath in nitrogen atmosphere and stirred for an additional 1 hr at the same temp and for 2·5 hr at room temp. To this mixture was successively added water (0·15 ml), 3N NaOH (0·3 ml) and 30% H_2O_2 (0·3 ml) and stirred for 1 hr at room temp. The mixture was poured onto ice water, extracted with chloroform, washed with water, 2% Na_2SO_3 and water, dried (Na_2SO_4) and evaporated leaving a syrup (210 mg). The residue was chromatographed on neutral alumina (7 g) giving a crystalline substance (180 mg), which was acetylated by acetic anhydride–pyridine at room temp. The crude acetate (190 mg) was chromatographed on neutral alumina (6 g) giving a viscous oil (118 mg). The oil was saponified with 2% KOH–methanol giving a crystalline substance (98 mg). The residue was fractionally crystallized from ethyl acetate giving carneagenin (XIV, 18 mg), colourless prisms, m.p. 262–264°, $[\alpha]_D^{25}$ −71·6° (±4°) (*c*, 0·391, methanol-chloroform (1:1)) (Found: C, 72·25; H, 9·90. $C_{27}H_{44}O_5$ requires: C, 72·28; H, 9·89%) and colourless needles (30 mg), m.p. 242–244° (Found: C, 72·73; H, 10·14. $C_{27}H_{44}O_5$ requires: C, 72·28; H, 9·89%), identical with isocarneagenin (XIIIa) by mixed m.p. and I.R. comparison.

Convertion of isocarneagenin (XIIIa) *into isorhodeasapogenin* (IIIa)

Tosyl chloride (10 mg) was added to a solution of isocarneagenin (XIIIa, 13 mg) in pyridine (1 ml) and left for 16 hr at room temp. A solution of the crude tosylate obtained (15 mg) in dry tetrahydrofuran (3 ml) was added to a suspension of lithium aluminum hydride (20 mg) in dry tetrahydrofuran (1 ml), refluxed with stirring for 3 hr, decomposed by addition of water, extracted with chloroform, washed with water, dried (Na_2SO_4) and evaporated leaving a syrup (13 mg). The residue was chromatographed on alumina giving the starting material (XIIIa, 6 mg) and colourless needles (5 mg), m.p. 235–238°, identical with isorhodeasapogenin (IIIa) by mixed m.p. and I.R. comparison.

Isomerization of carneagenin (XIV) *to isocarneagenin* (XIIIa)

A solution of carneagenin (XIV, 3 mg) in 4% HCl–methanol (2 ml) was allowed to stand for 1 hr at room temp, neutralized with 2N Na_2CO_3, extracted with chloroform, washed with water, dried ($NaSO_4$) and evaporated leaving a crystalline substance (3 mg). The residue was recrystallized from ethyl acetate giving isocarneagenin (XIIIa), colourless needles, m.p. 242–244°.

Isomerization of V *to* IV

IV (5 mg) was isomerized as described above to give V (5 mg), colourless prisms, m.p. 210–213° (from methanol).