Structures of Three New Steroidal Sapogenins from *Dioscorea prazeri*

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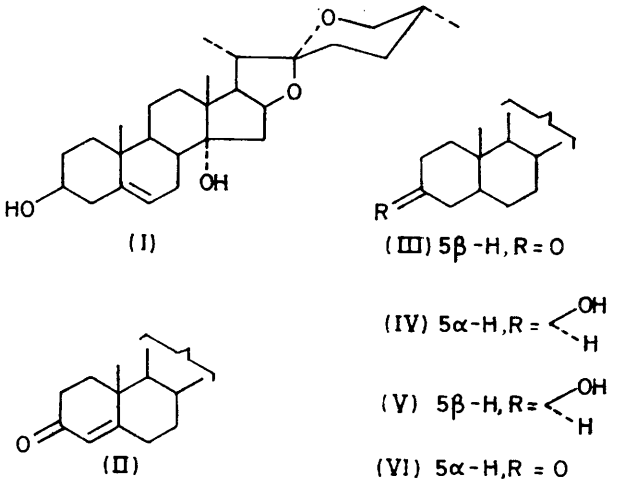
Three new steroidal sapogenins isolated from the rhizomes of *Dioscorea prazeri* have been shown on the basis of chemical and spectral evidence to be (25*R*)-spirost-5-en-3β,14-diol (I), (25*R*)-14-hydroxyspirost-4-en-3-one (II), and (25*R*)-14-hydroxy-5β-spirostan-3-one (III).

FROM the petroleum extract of *Dioscorea prazeri* we have isolated three new substances which gave typical steroidal colour reactions, showed i.r. absorptions characteristic of spirostanols [1] of the *iso*-series and gave mass spectral fragments (*a*—*c*) associated with spirostanols.[2] They are therefore steroidal sapogenins and have been designated as prazerigenins A—C.

Prazerigenin A, $C_{27}H_{42}O_4$, formed a monoacetate whose i.r. spectrum showed a band due to a free hydroxy-group, which was lost as water on treatment with phosphoryl chloride–pyridine. The genin gave a positive test for unsaturation with tetranitromethane. Oppenauer oxidation yielded an αβ-unsaturated ketone (u.v. and i.r.), and oxidation with chromic oxide gave a product with u.v. absorption typical of a steroidal Δ^4-3,6-dione.[3] On treatment with t-butyl chromate the acetate gave an αβ-unsaturated ketone (Δ^5-7-one), as would be expected of compounds of the cholesterol acetate type.[4] These results led to the conclusion that the genin has a 3-hydroxy-group and a 5,6-double bond. The tertiary hydroxy-group could be at one of positions 8, 9, 14, 16, 17, 20, and 25. Position 25 was ruled out by the presence of the fragments *a*—*c* in the mass spectrum and of a three-proton n.m.r. doublet at δ 0.79 characteristic of $C(27)H_3$.[5] Position 20 was ruled out by the observation of a $C(21)H_3$ signal as a doublet at δ 0.97. Positions 20 and 25 were also ruled out by the spectral properties of the (two) dehydration products of the acetate. A 20-hydroxy-compound would on dehydration have given a $\Delta^{20(21)}$ compound,[6] which would be recognisable by the i.r. absorption characteristic of an exocyclic methylene group. A 25-hydroxy-compound would be expected to give two isomers (Δ^{24}- and Δ^{25}-) both having a CH_3·C= group.[7] The two anhydro-acetates did not show evidence for either of the above-mentioned features. Position 17 was ruled out since our compound would then be identical with pennogenin, whereas direct comparison showed that they were different. Position 16 was ruled out since the 16α-proton n.m.r. signal was present at the characteristic[8] position δ 4.63. The choice thus lay among 8, 9, and 14. By comparison of the n.m.r. δ values of the 13 and 10-Me in *O*-acetylprazerigenin A with those calculated on the basis of Tables furnished by Tori *et al.*,[9] the OH was assigned to the 14α-position. On the basis of this structure, two anhydro-derivatives would be expected, namely $\Delta^{8(14)}$ and Δ^{14}. The former would have no additional vinylic proton in comparison with the parent genin, while the latter would have one, at C-15. In fact the genin acetate yielded two anhydro-compounds of which one but not the other contained an additional vinylic proton. The genin was catalytically reduced to remove the double bond and then acetylated. The product was identical with authentic 14α-hydroxytigogenin acetate [10] (m.p., $[\alpha]_D$, and i.r. spectrum). Hence prazerigenin A is (25*R*)-spirost-5-en-3β,14-diol (I).



Prazerigenin B, $C_{27}H_{40}O_4$, possessed an αβ-unsaturated CO grouping (u.v., i.r.) and also a hydroxy-group resistant to acylation. It was identical with the Oppenauer oxidation product of genin A and is therefore identified as (25*R*)-14-hydroxyspirost-4-en-3-one (II).

Prazerigenin C, $C_{27}H_{42}O_4$, has a ketone group in a six-membered ring (i.r.), no unsaturation, and a hydroxy-group resistant to acylation. The ketone was assigned to position 3 since the genin was identical with the product of catalytic reduction of prazerigenin B under

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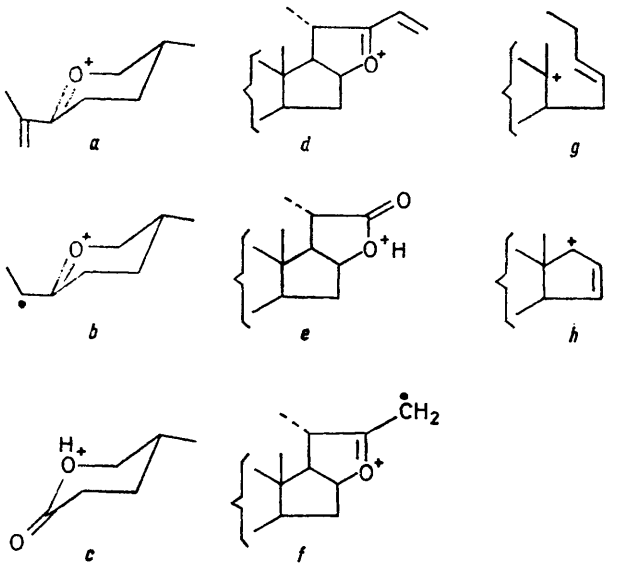
alkaline conditions.[11] The stereochemistry at C-5 was deduced as β from the observation that prazerigenin C was different from 14α-hydroxytigogenone obtained by oxidation by chromic oxide of dihydroprazerigenin A (5α-H). Further, when prazerigenin C was reduced with sodium borohydride and the resulting 3β-alcohol was acetylated, the product was different from the acetate of dihydroprazerigenin A. On the basis of these observations, prazerigenin C would be (25*R*)-14-hydroxy-5β-spirostan-3-one (III). The n.m.r. data were fully in agreement with this structure.



Fragments *d–h*, when present, have the following substituents/unsaturation: 3β-OH, Δ^{5}-, 14α-OH when derived from (I); 3-oxo, Δ^{4}-, 14α-OH from (II); 3-oxo-5β-H, 14α-OH from (III), 3-oxo-5α-H, 14α-OH from (VI), 3β-OAc-5β-H, 14α-OH from (V) acetate; 3β-OAc, $\Delta^{5,8(14)}$- and 3β-OAc, $\Delta^{5,14}$- from the two anhydro-compounds.

EXPERIMENTAL

M.p.s were taken on a Kofler hot-stage apparatus. Optical rotations were taken for solutions in chloroform. U.v. spectra were recorded for methanolic solutions on a Hilger-Uvispek spectrophotometer. I.r. spectra were recorded on a Perkin-Elmer Infracord 137 instrument for KBr discs. N.m.r. spectra were taken for solutions in [^{2}H]chloroform except where otherwise stated, with tetramethylsilane as internal standard, on a Varian A-60 instrument. All compounds were tested for purity by t.l.c. on silica gel.

Isolation.—The petroleum extract (6 g) obtained from the rhizomes (2.5 kg) of *Dioscorea prazeri* was taken up in chloroform and an excess of methanol was added. The precipitated solid was purified in the same manner and the resulting colourless solid (2.0 g) was chromatographed over silica gel. The initial benzene–ethyl acetate (48 : 2) eluates yielded prazerigenin C (25 mg) and later eluates yielded prazerigenin B (50 mg). Benzene–ethyl acetate (47 : 3) eluted prazerigenin A (1.6 g).

Prazerigenin A [(25R)-*spirost-5-en*-3β,14-*diol*] had m.p. 220—222°; $[\alpha]_D$ −84.9° (*c* 0.73); R_F 0.30 (benzene–ethyl acetate–methanol, 95 : 5 : 4) (Found: C, 75.0; H, 10.1. $C_{27}H_{42}O_4$ requires C, 75.3; H, 9.8%); $\nu_{max.}$ 3 448, 3 000, 1 648, 982, 920, 900, 867, 842, and 820 cm^{-1} (900 cm^{-1} band stronger than that at 920 cm^{-1}); *m/e* 430 (M^+, 2%). 412 (M − H_2O, 100), 394 (M − $2H_2O$, 3), 379 (394 − Me, 23), 371 (*d*, 2), 361 (*e*, 3), 358 (*f*, 3), 353 (*d* − H_2O, 4), 343 (*e* − H_2O, 3), 340 (*f* − H_2O, 9), 316 (*g*, 2), 298 (*g* − H_2O 13), 287 (*h*, 1), 283 (*g* − H_2O − Me, 6), 280 (*g* − $2H_2O$, 5), 269 (*h* − H_2O, 6), 265 (*g* − $2H_2O$ − Me, 12), 251 (*h* − $2H_2O$, 6), 139 (*a*, 93), 126 (*b*, 30), and 115 (*c*, 16); *acetate* (Ac_2O–C_5H_5N; 37 °C; 18 h), m.p. 155—158°; $[\alpha]_D$ −150.6° (*c* 0.81); R_F 0.76 (benzene–ethyl acetate–methanol, 95 : 5 : 3) (Found: C, 73.9; H, 9.6. $C_{29}H_{44}O_5$ requires C, 73.7; H, 9.3%); $\nu_{max.}$ 3 472, 2 914, 1 718, 1 627, 1 235, 980, 917, 897, 870, 841, and 823 cm^{-1}; δ 5.43 (1 H, m, 6-H), 4.63 (2 H, m, 16- and 3-H), 3.50 (2 H, m, 26-H), 2.04 (Ac), 1.05 (3 H, s, 10-Me; calc. 1.03), 0.97 (3 H, d, *J* 7 Hz, 21-H_3), 0.92 (3 H, s, 13-Me; calc. 0.91), and 0.79 (3 H, d, *J* 7 Hz, 27-H_3); δ (C_5D_5N) 5.36 (1 H, m, 6-H), 4.68 (2 H, m, 16- and 3-H), 3.48 (2 H, m, 26-H), 2.00 (Ac), 1.13 (3 H, d, *J* 8 Hz, 21-H_3), 1.06 (6 H, s, 13- and 10-Me; calc. 13-Me 1.08, 10-Me 1.04), and 0.66 (3 H, d, *J* 6 Hz, 27-H_3).

Oppenauer oxidation of prazerigenin A: formation of the Δ^4-3-*one* (II). A solution of prazerigenin A (0.05 g) and aluminium isopropoxide (0.05 g) in anhydrous toluene (5 ml) was heated to boiling, cyclohexanone (1 ml) was added, and the mixture was refluxed for 10 h. The excess of cyclohexanone was removed by steam distillation and the residue was acidified and extracted with ether. The extract was evaporated and the residue crystallised from chloroform–methanol as *needles* (0.03 g), m.p. 244—247°; R_F 0.40 (benzene–ethyl acetate–methanol, 95 : 5 : 4) (Found: C, 75.5; H, 9.6. $C_{27}H_{40}O_4$ requires C, 75.7; H, 9.3%); $\lambda_{max.}$ 240 nm (log ε 4.13); $\nu_{max.}$ 3 505, 1 670, 1 602, 980, 920, 900, and 862 cm^{-1}.

(25R)-14-*Hydroxyspirost-4-en-3,6-dione from prazerigenin A*. A solution of prazerigenin A (0.05 g) in acetone (10 ml) was treated with Jones reagent (containing 25 mg of CrO_3) at room temperature for 2 h. The product, isolated in the usual manner, was purified by passage through a column of silica gel and crystallised from chloroform–petroleum as light yellow *plates* (0.025 g), m.p. 247—250°; $[\alpha]_D$ −37.5° (*c* 0.96); R_F 0.39 (benzene–ethyl acetate–methanol, 95 : 5 : 4) (Found: C, 72.8; H, 9.0. $C_{27}H_{38}O_5$ requires C, 73.3; H, 8.6%); $\lambda_{max.}$ 254 nm (log ε 3.99); $\nu_{max.}$ 3 490, 1 680, 1 670, 1 595, 978, 918, 900, and 865 cm^{-1}.

7-*Oxoprazerigenin A acetate*. A solution of prazerigenin A acetate (0.1 g) in carbon tetrachloride (2 ml), acetic acid (1 ml), and acetic anhydride (0.25 ml) was treated at 55—60 °C with a solution containing t-butyl chromate [12] in carbon tetrachloride (0.15 g in 2 ml), acetic acid (0.5 ml), and acetic anhydride (0.25 ml) during 45 min with stirring. Stirring was continued for 10 h at 65 °C, the mixture was cooled to 20 °C and the excess of oxidant was destroyed by adding aqueous 10% oxalic acid (2 ml) during 1 h. The product was isolated by adding water and extracting with chloroform, and crystallised from chloroform–methanol as *plates* (0.06 g), m.p. 255—257°, $[\alpha]_D$ −111.4° (*c* 0.79); R_F 0.64 (benzene–ethyl acetate–methanol, 95 : 5 : 3) (Found: C, 71.9; H, 9.0. $C_{29}H_{42}O_6$ requires C, 71.6; H, 8.7%); $\lambda_{max.}$ 234 nm (log ε 4.13); $\nu_{max.}$ 3 500, 1 735, 1 660, 1 610, 1 240, 982, 922, 900, and 868 cm^{-1}.

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Action of phosphoryl chloride on prazerigenin A acetate. Prazerigenin A acetate (0.15 g) in dry pyridine (4 ml) was treated with phosphoryl chloride (0.5 ml) at 0 °C and left at 0 °C for 0·5 h and then at room temperature overnight. The product isolated in the usual way was separated into two components by preparative t.l.c. (benzene–ethyl acetate, 95 : 5). (25R)-*Spirosta*-5,8(14)-*dien*-3β-*yl acetate* had m.p. 181—184°, $[\alpha]_D$ −133.3° (*c* 0.78) (yield 70 mg); R_F 0.66 (benzene–ethyl acetate, 95 : 5) (Found: C, 76.5; H, 9.6. $C_{29}H_{42}O_4$ requires C, 76.6; H, 9.3%); $\nu_{max.}$ 2 941, 1 724, 1 235, 980, 924, 903, 868, 851, and 816 cm^{-1}; δ 5.37 (1 H, m, 6-H), 4.50 (2 H, m, 16- and 3-H), 3.53 (2 H, m, 26-H), 2.04 (Ac), 1.02 (3 H, s, 13-Me; calc. 0.97), 0.97 (3 H, d, *J* 6 Hz, 21-H_3), 0.92 (3 H, s, 10-Me; calc. 0.92), and 0.78 (3 H, d, *J* 6 Hz, 27-H_3); *m/e* 454 (M^+, 8%), 395 (*d*, 36), 394 (*M* − AcOH, 100), 379 (394 − Me, 10), 340 (*g*, 1), 325 (*e* − AcOH, 8), 322 (*f* − AcOH, 6), 311 (*h*, 4), 280 (*g* − AcOH, 13), 265 (*g* − AcOH − Me, 22), 251 (*h* − AcOH, 22), 139 (16), 126 (7), and 115 (10). (25R)-*Spirosta*-5,14-*dien*-3β-*yl acetate* had m.p. 200—203°; $[\alpha]_D$ −17.8° (*c* 0.90) (yield 40 mg); R_F 0.55 (benzene–ethyl acetate, 95 : 5) (Found: C, 76.8; H, 9.6%); $\nu_{max.}$ 2 950, 1 725, 1 637, 1 235, 977, 917, 899, 864, 838, and 814 cm^{-1}; δ 5.42 (2 H, m, 6- and 15-H), 4.95 (1 H, double d, 16-H, $J_{15,16}$ 8, $J_{16,17}$ 3 Hz), 4.58 (1 H, m, 3-H), 3.53 (2 H, m, 26-H), 2.08 (Ac), 1.07 (6 H, s, 13- and 10-Me; calc. 13-Me 1.04, 10-Me 1.03), 1.02 (3 H, d, *J* 7 Hz, 21-H_3), and 0.82 (3H, d, *J* 6 Hz, 27-H_3); *m/e* 454 (M^+, 4%), 395 (*d*, 32), 394 (*M* − AcOH, 100), 379 (394 − Me, 5), 340 (*g*, 9), 325 (*e* − AcOH, 5), 322 (*f* − AcOH, 19), 280 (*g* − AcOH, 34), 265 (*g* − AcOH − Me, 12), 139 (21), 126 (15), and 115 (12).

5,6-*Dihydroprazerigenin* (14α-*Hydroxytigogenin*) (IV).—This was prepared by hydrogenating prazerigenin A (0.1 g) in glacial acetic acid (5 ml) over palladium–charcoal (10%; 0.03 g) for 2 h at atmospheric pressure and room temperature. It crystallised from chloroform–methanol as plates (0.09 g), m.p. 216—218°; $[\alpha]_D$ −66.4° (*c* 0.86); R_F 0.28 (benzene–ethyl acetate–methanol, 95 : 5 : 4) (Found: C, 75.2; H, 10.5. Calc. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.2%) (lit.,[10] m.p. 211—212°, $[\alpha]_D$ −58.8°); *acetate* (Ac_2O–C_5H_5N; 37 °C; 18 h), m.p. 192—195°; $[\alpha]_D$ −65.0° (*c* 0.96); R_F 0.72 (benzene–ethyl acetate–methanol, 95 : 5 : 3) (Found: C, 73.1; H, 9.9. Calc. for $C_{29}H_{46}O_5$: C, 73.4; H, 9.7%); $\nu_{max.}$ 3 580, 1 739, 1 242, 980, 920, 899, and 870 cm^{-1} (i.r. spectrum identical with that of authentic 14α-hydroxytigogenin acetate) (lit.,[10] m.p. 187—189°, $[\alpha]_D$ −60.8°).

(25R)-14-*Hydroxy*-5α-*spirostan*-3-*one*. An ice-cold solution of compound (IV) (0.03 g) in pyridine (1 ml) was treated with chromic oxide (0.03 g) in pyridine (1 ml) and left at room temperature for 18 h. The crude product was chromatographed over silica gel and crystallised from acetone–petroleum as *needles* (0.017 g), m.p. 226—228°; $[\alpha]_D$ −45.3° (*c* 1.06); R_F 0.49 (benzene–ethyl acetate–methanol, 95 : 5 : 4) (Found: C, 75.2; H, 10.0. $C_{27}H_{42}O_4$ requires C, 75.3; H, 9.8%); $\nu_{max.}$ 3 571, 1 709, 980, 922, 897, and 866 cm^{-1}; *m/e* 430 (M^+, 54%), 412 (*M* − H_2O, 25), 402 (*M* − CO, 14), 397 (*M* − H_2O − Me, 10), 371 (*d*, 18), 361 (*e*, 13), 358 (*f*, 48), 316 (*g*, 36), 298 (*g* − H_2O, 100), 287 (*h*, 11), 283 (*g* − H_2O − Me, 34), 269 (*h* − H_2O, 65), 139 (90), 126 (35), and 115 (30).

Prazerigenin B [(25R)-14-*hydroxyspirost*-4-*en*-3-*one*] had m.p. 245—247°; $[\alpha]_D$ −43.3° (*c* 0.60); $\lambda_{max.}$ 242 nm (log ε 4.11); $\nu_{max.}$ 3 500, 1 672, 1 602, 978, 920, 900, and 862 cm^{-1}; *m/e* 428 (M^+, 9%), 410 (*M* − H_2O, 6), 400 (*M* − CO, 1.5), 395 (410 − Me, 1), 369 (*d*, 9), 359 (*e*, 8), 356 (*f*, 16), 351 (*d* − H_2O, 2), 338 (*f* − H_2O, 1.5), 314 (*g*, 21), 296 (*g* − H_2O, 88), 285 (*h*, 3), 281 (*g* − H_2O − Me, 19), 267 (*h* − H_2O, 18), 139 (100), 126 (25), and 115 (12), and was identical with the Oppenauer oxidation product of prazerigenin A described earlier (mixed m.p., t.l.c., and i.r. spectrum).

Catalytic Reduction of Prazerigenin B.—Prazerigenin B (II) (0.025 g) in dry ethanol (3 ml) was treated with potassium hydroxide (0.015 g) in dry ethanol (3 ml) and shaken in hydrogen with palladium–charcoal (10%; 0.01 g) for 2 h at room temperature and atmospheric pressure. The mixture was filtered and the filtrate poured into water; the solid was filtered off and crystallised from acetone–petroleum as needles (0.015 g), m.p. 237—240°, identical with prazerigenin C, described below (mixed m.p., t.l.c., and i.r. spectrum).

Prazerigenin C [(25R)-14-*hydroxy*-5β-*spirostan*-3-*one*] (III) had m.p. 237—240°; $[\alpha]_D$ −48.3° (*c* 1.16); R_F 0.42 (benzene–ethyl acetate–methanol, 95 : 5 : 4) (Found: C, 75.2; H, 9.8. $C_{27}H_{42}O_4$ requires C, 75.3; H, 9.8%); $\nu_{max.}$ 3 597, 1 701, 980, 922, 899, and 870 cm^{-1}; δ 4.61 (1 H, m, 16-H), 3.43 (2 H, m, 26-H), 1.05 (3 H, s, 10-Me; calc. 1.04), 0.97 (3 H, d, *J* 7 Hz, 21-H_3), 0.92 (3 H, s, 13-Me; calc. 0.92), and 0.78 (3 H, d, *J* 7 Hz, 27-H_3); *m/e* 430 (M^+, 17%), 412 (*M* − H_2O, 6), 402 (*M* − CO, 4), 397 (412 − Me, 0.5), 371 (*d*, 5), 361 (*e*, 2), 358 (*f*, 12), 353 (*d* − H_2O, 0.5), 343 (*e* − H_2O, 1), 340 (*f* − H_2O, 0.5), 316 (*g*, 20), 298 (*g* − H_2O, 54), 287 (*h*, 1), 283 (*g* − H_2O − Me, 7), 269 (*h* − H_2O, 8), 139 (100), 126 (31), and 115 (16).

Reduction of prazerigenin C. To a solution of prazerigenin C (III) (0.02 g) in methanol (3 ml) sodium borohydride (0.03 g) was added and the solution was set aside for 2 h. The product, isolated in the usual manner, was a mixture of two compounds (t.l.c.) in the ratio *ca.* 9 : 1, but they could not be separated because of close R_F values. The mixture was acetylated (acetic anhydride–pyridine) at 37 °C for 18 h. The compounds were separated by preparative t.l.c. over silver nitrate-impregnated silica gel. The minor product was amorphous. The major product, (25R)-5β-*spirostan*-3β,14-*diol* (V), crystallised from chloroform–methanol as needles (0.012 g), m.p. 189—191°; $[\alpha]_D$ −76.7° (*c* 0.91); R_F 0.77 (benzene–ethyl acetate–methanol, 95 : 5 : 3) (Found: C, 73.8; H, 9.8. $C_{29}H_{46}O_5$ requires C, 73.4; H, 9.7%); $\nu_{max.}$ 3 600, 1 730, 1 250, 980, 920, 900, and 866 cm^{-1}; *m/e* 474 (M^+, 6%), 456 (*M* − H_2O, 5), 415 (*d*, 6), 405 (*e*, 1), 402 (*f*, 14), 360 (*g*, 6), 342 (*g* − H_2O, 69), 327 (*g* − H_2O − Me, 22), 313 (*h* − H_2O, 5), 139 (100), 126 (56), and 115 (19).

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