EPI-NEOTIGOGENIN AND EPI-TIGOGENIN, TWO NEW STEROIDAL SAPOGENINS FROM *CORDYLINE CANNIFOLIA* LEAVES

GERALD BLUNDEN and JAFFER A. JAFFER

School of Pharmacy, Portsmouth Polytechnic, King Henry I Street, Portsmouth, P01 2DZ, U.K.

and

KENNETH JEWERS

Tropical Products Institute, 56/62 Gray's Inn Road, London, WC1X 8LU, U.K.

and

WILLIAM J. GRIFFIN

Department of Pharmacy, University of Queensland, St. Lucia, Queensland 4067, Australia

ABSTRACT.—From the monohydroxy steroidal sapogenin fraction of *Cordyline cannifolia* R. Br. leaves, 3-*epi*-neotigogenin (1a) and 3-*epi*-tigogenin (1b) were isolated and characterized from tlc, ir, nmr and mass spectral data and by conversion into known spirostanes. Neither compound has been isolated previously from a plant source. In addition, yamogenin and diosgenin were found. Tlc methods are described for the separation of 3α- and 3β-monohydroxy spirostanes.

From the leaves of *Cordyline cannifolia* R. Br. cordylagenin [(25*S*)-5α-spirostan-1β,3α-diol], its 25*R* epimer cannigenin, brisbagenin [(25*R*)-5α-spirostan-1β,3β-diol] and yamogenin [(25*S*)-spirost-5-en-3β-ol] have been isolated (1–3). The presence of ruscogenin [(25*R*)-spirost-5-en-1β,3β-diol] was indicated also on the basis of chromatographic evidence (3). Also isolated was another sapogenin which had the same chromatographic characteristics as smilagenin [(25*R*)-5β-spirostan-3β-ol] in the development systems used and which had a mass spectrum identical with that of smilagenin. However, the melting point of the isolated sapogenin was higher than that obtained for smilagenin (3). Insufficient material was available to characterize the compound further at that time. Because of this, the monohydroxy sapogenins obtained from the leaves of *C. cannifolia* have been studied further, and the results are reported in this paper.

MATERIALS AND METHODS

EXTRACTION OF SAPOGENINS.—*Cordyline cannifolia* R. Br. leaves, collected from Davis Creek and Tinaroo Road Creek, Queensland[1], were oven dried at 60° and powdered. The powdered leaf (1.3 kg) was initially depigmented with chloroform for 2 hr before the sapogenins were extracted by the method of Blunden *et al.* (4). This entailed soaking the plant material in water for 24 hr at room temperature, refluxing with 2*N* hydrochloric acid for 2 hr, separation of the acid-insoluble material by filtration, and washing with water and ammonium hydroxide solution and then with water until neutral. The dried, acid-insoluble residue was extracted with chloroform for 6 hr.

DETECTION AND ISOLATION OF SAPOGENINS.—The crude sapogenin extract was examined by two-dimensional tlc on air-dried silica gel G layers (wet thickness 250 mμ) using dichloromethane-methanol-formamide (93:6:1) in the first direction (system I) and two-fold development in cyclohexane-ethyl acetate-water (600:400:1) in the second (system II). The steroidal compounds were located by spraying with 50% sulfuric acid and heating at 100° until the characteristic colors developed (5).

To separate the monohydroxy from most of the dihydroxy sapogenins, the extract was chromatographed on a column of silica gel, 60–120 mesh (B.D.H. Chemicals Ltd.), using successively chloroform, chloroform-ethanol (99:1), chloroform-ethanol (49:1), chloroform-ethanol (97:3) and ethanol. The collected fractions were screened for sapogenins by tlc using system I. Chloroform and chloroform-ethanol (99:1) did not afford any sapogenin, chloroform-

[1]Herbarium Australiense voucher numbers HN.VKM 1600 and HN.VKM 986.

ethanol (49:1) and chloroform-ethanol (97:3) eluted the monohydroxy sapogenins, along with a small quantity of dihydroxy compounds, and ethanol furnished only dihydroxy compounds.

The chloroform-ethanol (49:1) and chloroform-ethanol (97:3) fractions were bulked and concentrated, and system I was used to separate the monohydroxy sapogenins from the dihydroxy compounds by preparative tlc on air-dried, silica gel G layers (wet thickness 500 mμ). The chromatograms were sprayed with distilled water to locate the sapogenin bands. The monohydroxy sapogenin bands were dried, removed together and eluted with chloroform. The extract was purified further by preparative tlc by system II.

The purified monohydroxy sapogenin extract was separated into its individual components by preparative tlc. System I was used to divide the extract into fractions A and B. Fraction A was subdivided further into A1 and A2 by means of three-fold development in *n*-hexane-ethyl acetate (4:1) (system III). Fractions A–1 and B were each separated into two components, A–11 and A–12, and B–1 and B–2, respectively, on layers of silica gel G containing 2% silver nitrate. The layers, 500 mμ wet thickness, had been activated at 100° for 1 hr prior to use (6). The chromatograms were developed three times in dichloromethane-acetone (49:1) (system IV).

PREPARATION OF SPIROSTANES.—Smilagenin and tigogenin [(25*R*)-5α-spirostan-3β-ol] were purchased from Steraloids Ltd. Hecogenin [3β-hydroxy-(25*R*)-5α-spirostan-12-one] and neotigogenin [(25*S*)-5α-spirostan-3β-ol] were isolated from the leaves of *Agave sisalana* Perrine, and yamogenin was isolated from the seeds of *Trigonella foenum-graecum* L. Diosgenin [(25*R*)-spirost-5-en-3β-ol] was given by Dr. J. Barbour; 3-*epi*-diosgenin and 3-*epi*-sceptrumgenin [spirost-5,25(27)-diene-3α-ol] was provided by Dr. Shigeo Nozoe; and sarsasapogenin [(25*S*)-5β-spirostan-3β-ol] was supplied by Ir. L. E. Miramontes C.

A. 3-*Epi*-SARSASAPOGENIN AND 3-EPI-SMILAGENIN.—Sarsasapogenin was treated with Jones' reagent to produce sarsasapogenone, which reacted with sodium borohydride in methanol to give a mixture of sarsasapogenin and 3-*epi*-sarsasapogenin. The two compounds were separated by preparative tlc using system III. Starting with smilagenin, 3-epi-smilagenin was produced by the same route.

B. 3-*Epi*-TIGOGENIN.—Tigogenin was treated with Jones' reagent to give tigogenone. This was hydrogenated for 6 hr over Adams' catalyst in glacial acetic acid containing 2% hydrochloric acid (12) to give a mixture of tigogenin acetate and 3-*epi*-tigogenin acetate. Hydrolysis with 5% methanolic potassium hydroxide afforded tigogenin and 3-*epi*-tigogenin, which were separated by preparative tlc using system III.

C. 3-*Epi*-HECOGENIN.—Hecogenin (3.64 g) in ethylene glycol (50 ml) was treated with boron trifluoride-ether complex (8 ml). After 16 hr at room temperature, the solution was extracted with chloroform, the chloroform layer was separated and washed with water until neutral. Evaporation of the solvent gave a white solid which, after recrystallization from acetone, gave crystals of 12-ethylene dioxy-(25*R*)-spirostan-3β-ol, mp[2] 215–217°. Its ir spectrum[3] showed absorption at 3524 cm^{-1} (hydroxyl group), at 1179, 1125 and 1051 cm^{-1} (ethylene ketal function) and at 981, 924, 900 and 862 cm^{-1} (spiroketal moiety). The strong absorption at 1714 cm^{-1} (carbonyl group) present in the ir spectrum of hecogenin was absent. The nmr spectrum[4] confirmed the conversion into 12-ethylene dioxy-(25*R*)-spirostan-3β-ol.

A solution of 12-ethylene dioxy-(25*R*)-spirostan-3β-ol (1.98 g) in pyridine (60 ml) was added slowly with stirring to a previously prepared solution of chromium trioxide (1.40 g) in pyridine (60 ml) (13). The mixture was kept at 37° for 4 hr and then at room temperature for 48 hr, by which time the reaction was complete (tlc). Water (360 ml) was added to the reaction mixture, which was extracted with 6 x 100 ml ether. After the extract was washed with water, the ether was dried (magnesium sulphate) and evaporated to yield a yellowish, crystalline solid which, after crystallization from acetone, gave silky, thin needles of 12-ethylene dioxy-(25*R*)-spirostan-3-one, mp 229°. The ir spectrum showed absorption at 1714 cm^{-1} (carbonyl group), but the strong absorption at 3524 cm^{-1} (hydroxyl group) present in the ir spectrum of 12-ethylene dioxy-(25*R*)-spirostan-3β-ol was absent. The nmr spectrum was consistent with 12-ethylene dioxy-(25*R*)-spirostan-3-one.

12-Ethylene dioxy-(25*R*)-spirostan-3-one was hydrogenated for 4 hr over Adams' catalyst in glacial acetic acid containing 2% hydrochloric acid (12). Three major products were formed, two of which were hecogenin acetate and 3-*epi*-hecogenin acetate. After hydrolysis with 5% methanolic potassium hydroxide solution, 3-*epi*-hecogenin was isolated by preparative tlc. Crystallization from acetone yielded needles, mp 216°, (M^+, *m/e* 430). Its ir spectrum showed absorption at 3410 cm^{-1} (hydroxyl group), 1714 cm^{-1} (carbonyl group) and 982, 919, 896 and 867 cm^{-1} (spiroketal moiety), with the absorption at 896 cm^{-1} greater than at 919 cm^{-1} (25*R*-spirostane). We have not found any previous record of this compound.

---

[2]Mp were determined on a Kofler Block and are uncorrected.

[3]Infrared spectra were measured in potassium bromide discs.

[4]Nmr spectra were determined in $CDCl_3$.

TLC SEPARATION OF 3α- AND 3β-MONOHYDROXY SPIROSTANES.—All the reference monohydroxy spirostanes available were chromatographed on air-dried silica gel G layers, 250 μm wet thickness, using system III, and on activated layers of silica gel G containing 2% silver nitrate, 250 μm wet thickness, using system IV.

RESULTS AND DISCUSSION

After preliminary extraction of *C. cannifolia* leaves to remove pigments, the saponins were hydrolyzed and the liberated sapogenins extracted. The extract, on examination by two-dimensional tlc, produced 5 spots, which were labelled A to E in order of decreasing R_f value. Earlier work has shown that spot C is composed of cannigenin and cordylagenin, spot D of ruscogenin and spot E of brisbagenin (1–3). Column chromatography and preparative tlc were used to separate the compounds forming spots A and B from the dihydroxy compounds forming spots C, D and E. By preparative tlc, the compounds forming spots A and B were divided into 5 fractions, A–11, A–12, A–2, B–1 and B–2.

Compound A–11, $C_{27}H_{44}O_3$, (M^+, *m/e* 416.331; calculated 416.329), mp 190–191°, crystallized as needles from methanol. Its ir spectrum showed absorption at 3320 cm^{-1} (hydroxyl group) and at 989, 921, 902 and 852 cm^{-1} (spiroketal moiety); the absorption at 921 cm^{-1} was of greater intensity than that at 902 cm^{-1} (25*S*-spirostane). The mass spectrum showed strong ions at *m/e* 344, 302, 287, 273, 255 and 139, which are characteristic of saturated monohydroxy spirostanes. In the nmr spectrum, the resonances of the secondary and tertiary methyl groups were unresolved and were observed as broad singlets at δ 0.72 and δ 0.76. A signal at δ 4.02 (1H, m, $W_{1/2} \simeq 8.0$ Hz) was assigned to a hydroxy group at C–3 with an axial conformation (1, 3, 7, 8). Jones' oxidation of A–11 gave a compound mp 215° (literature value (9) for neotigogenone 211–214°), the ir spectrum of which showed strong absoprtion at 1715 cm^{-1} (carbonyl group). This compound was reduced with sodium borohydride in methanol to give neo-tigogenin (tlc, mp, ir). From all the data obtained, A–11 was concluded to be 3-*epi*-neotigogenin (fig. 1a).

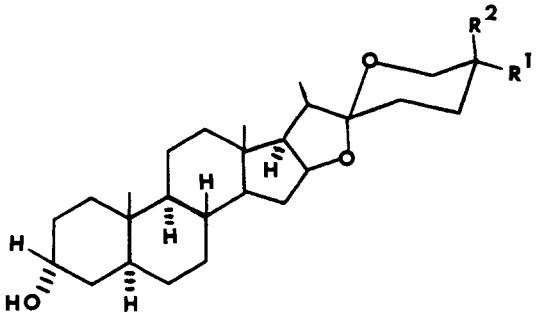

FIG. 1. 3α-Monohydroxy sapogenins from *Cordyline cannifolia* leaves.
a = 3-*epi*-neotigogenin; R^1 = H; R^2 = Me
b = 3-*epi*-tigogenin; R^1 = Me; R^2 = H

Compound A–12, $C_{27}H_{44}O_3$, (M^+, *m/e* 416.336; calculated 416.329) was isolated in small quantity. Its ir spectrum was identical with that of 3-*epi*-tigogenin, showing absorption at 3430 cm^{-1} (hydroxyl group) and at 981, 918, 896 and 862 cm^{-1} (spiroketal moiety), with the absorption at 896 cm^{-1} being of greater intensity than at 918 cm^{-1} (25*R*-spirostane). In all tlc systems used, compound A–12 had the same mobility as 3-*epi*-tigogenin. The mass spectrum was very similar to that of compound A–11 and of other saturated monohydroxy spirostanes.

Jones' oxidation of A–12 afforded a compound which had tlc and ir spectral characteristics identical with those of tigogenone and which, on reduction with sodium borohydride in methanol, gave a compound with the same tlc and ir characteristics as tigogenin. Although insufficient compound A–12 was isolated to obtain further information, it was concluded to be 3-*epi*-tigogenin (fig. 1b).

To our knowledge, this is the first record of the isolation from a plant source of of 3-*epi*-neotigogenin and 3-*epi*-tigogenin, but the latter compound has been synthesized (10). In an earlier paper, we identified a sapogenin isolated from *C. cannifolia* leaves as smilagenin, on the basis of tlc, ir, and mass spectral data. although its mp was higher than that of smilagenin (3). The tlc systems described in that paper did not separate compounds A–11 and A–12 from each other or from smilagenin. Moreover, the mass spectra of all three compounds are very similar. It is highly probable that the material isolated in the earlier study was a

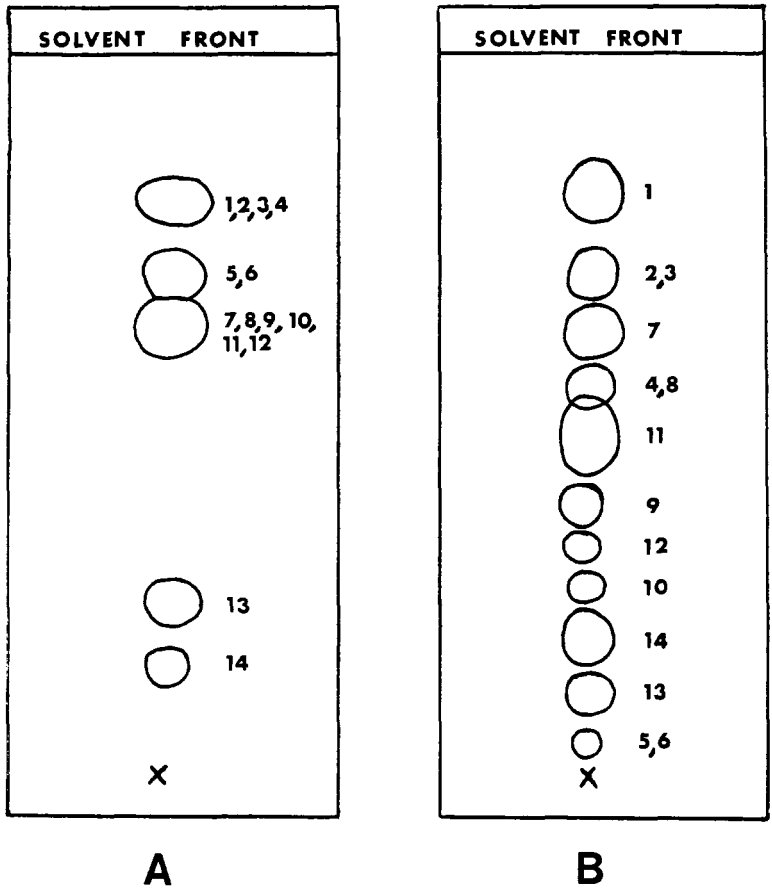

FIG. 2. Tlc separations of 3α- and 3β-monohydroxy spirostanes.

Adsorbent. A silica gel G; B. silica gel G, containing 2% silver nitrate.

Development solvents. A. three-fold in n-hexane-ethyl acetate (4:1); B. three-fold in dichloromethane-acetone (49:1).

Locating reagent. 50% sulfuric acid.

Spirostanes. 1=smilagenin; 2=sarsasapogenin; 3=3-*epi*-tigogenin; 4=3-*epi*-neotigogenin; 5=3-*epi*-diosgenin; 6=3-*epi*-sceptrumgenin; 7=tigogenin; 8=neotigogenin; 9=diosgenin; 10=yamogenin; 11=3-*epi*-smilagenin; 12=3-*epi*-sarsasapogenin; 13=3-*epi*-hecogenin; 14=hecogenin.

mixture of 3-*epi*-neotigogenin and 3-*epi*-tigogenin. As the ir spectrum of the isolated material was consistent with its being a 25*R*-sapogenin, it is probable that 3-*epi*-tigogenin predominated. It has been shown that the proportion of 25*R*- to 25*S*-sapogenins varies considerably between different samples of *C. cannifolia* leaves (1–3).

Compound A–2 was isolated in very small quantity as an oil. On tlc in system III, A–2 had the same mobility as 3-*epi*-diosgenin and 3-*epi*-sceptrumgenin; but in system IV, A–2 had a slightly higher R_f value than these two reference compounds. Moreover, it gave a yellow color with 50% sulfuric acid, whereas both 3-*epi*-diosgenin and 3-*epi*-sceptrumgenin gave purple colors. It was not possible to characterize the compound.

Compound B–1 crystallized as needles, mp 182° (literature value (11) for yamogenin 184–185°). Its tlc, ir and mass spectral characteristics were identical to those of yamogenin and, on hydrogenation over Adams' catalyst, a compound which had the same mobility as neo-tigogenin was formed. B–1 was identified as yamogenin, which we have isolated previously from *C. cannifolia* leaves (3).

B–2 was obtained in small quantity. As its tlc and ir characteristics were identical to those of diosgenin, this identity was assigned to the compound.

Earlier the 3-*epi*-neotigogenin and 3-*epi*-tigogenin mixture was erroneously identified as smilagenin because the three compounds had identical mobility in the developing solvents used (3). Therefore, a study of tlc systems that would resolve the 3α- and 3β-monohydroxy spirostanes was undertaken. Smilagenin, sarsasapogenin, 3-*epi*-tigogenin and 3-*epi*-neotigogenin are not resolved by system III. Also, 3-*epi*-diosgenin and 3-*epi*-sceptrumgenin are not separated from each other and tigogenin, neotigogenin, diosgenin, yamogenin, 3-*epi*-smilagenin and 3-*epi*-sarsasapogenin run together (fig. 2). More complete separation of the compounds is achieved by system IV, but neotigogenin and 3-*epi*-neotigogenin are not separated from each other. However, these two can be easily distinguished by system III. Sarsasapogenin and 3-*epi*-tigogenin are not resolved and neither are 3-*epi*-diosgenin and 3-*epi*-sceptrumgenin (fig. 2). The sulfuric acid locating reagent produced yellow colors with all the compounds with the exception of hecogenin and 3-*epi*-hecogenin, which turned yellowish-brown; diosgenin, 3-*epi*-diosgenin, yamogenin and 3-*epi*-sceptrumgenin became pinkish-purple.

ACKNOWLEDGMENTS

We wish to thank Dr. J. Barbour, Dr. Shigeo Nozoe and Ir. L. E. Miramontes C. for their kind gifts of reference sapogenins; Dr. J. B. Davis for the mass spectra; Mr. M. J. Nagler for the nmr spectra; and Mr. S. B. Mills and Miss B. Smith for technical assistance.

Received 2 April 1979.

LITERATURE CITED

1. K. Jewers, A. H. Manchanda, J. Dougan, M. J. Nagler, G. Blunden and W. J. Griffin, *Tetrahedron Lett.*, **1974**, 1475 (1974).
2. K. Jewers, M. B. Burbage, G. Blunden and W. J. Griffin, *Steroids*, **24**, 203 (1974).
3. W. J. Griffin, G. Blunden, K. Jewers, M. B. Burbage and M. J. Nagler, *Phytochemistry*, **15**, 1271 (1976).
4. G. Blunden, R. Hardman and W. R. Wensley, *J. Pharm. Pharmacol.*, **17**, 274 (1965).
5. R. D. Bennett and E. Heftmann, *J. Chromatogr.*, **9**, 353 (1962).
6. A. M. Dawidar, A. A. Saleh and S. L. Elmotei, *Planta Medica*, **24**, 367 (1973).
7. J. E. Bridgeman, P. C. Cherry, A. S. Clegg, J. M. Evans, Sir. E. R. H. Jones, A. Kasal, V. Kumar, G. D. Meakins, Y. Morisawa, E. E. Richards and P. D. Woodgate, *J. Chem. Soc. Sec. C.*, 1970, 250 (1970).
8. M. Takahira, Y. Kondo, G. Kusano and S. Nozoe, *Tetrahedron Lett.*, **1977**, 3647 (1977).
9. L. H. Goodson and R. C. Noller, *J. Amer. Chem. Soc.*, **61**, 2420 (1939).
10. R. E. Marker, *J. Amer. Chem. Soc.*, **62**, 2621 (1940).
11. R. Hardman and E. A. Sofowora, *Phytochemistry*, **9**, 645 (1970).
12. J. T. Edwards and J. M. Ferland, *Can. J. Chem.*, **44**, 1311 (1966).
13. G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, *J. Amer. Chem. Soc.*, **75**, 422 (1953).