Thin-layer chromatography of *Dioscorea* sapogenins

Paper chromatographic separations of steroidal sapogenins have been described by HEFTMANN AND HAYDEN[1], SANNIÉ AND LAPIN[2], MC.ALEER AND KOZLOWSKI[3] and WALL *et al*[4]. The procedures of HEFTMANN AND HAYDEN and SANNIÉ AND LAPIN are complicated by frequent lack of resolution due to tailing of spots at concentrations greater than 5 μg and by difficulties in maintaining stable chromatographic conditions[3]. The non-aqueous systems of MC. ALEER AND KOZLOWSKI and WALL *et al.* are technically more complicated and require several hours for development.

Thin-layer chromatography has been employed by several workers for the separation of steroidal sapogenins. SANDER[5–8] employed the technique for the separation of unknown sapogenins and in the identification of neotigogenin, tigogenin, diosgenin and gitogenin. TLC has been used by BENNETT AND HEFTMANN[9] for the separation of steroidal sapogenins using systems other than those described in this paper and with 50 % sulphuric acid as the detecting agent. SMITH AND FOELL[10] have given R_F values for C_{27} sapogenins with starch bound silica gel thin-layers, using hexane–ethyl acetate (4:1), hexane–ethyl acetate (1:1) and ethyl acetate as solvent systems and with phosphomolybdic acid among their detecting reagents. Spread-layer chromatography has also been applied to some sapogenins[11–13].

The chromatographic methods described in this paper were devised for the rapid separation of the components of the crude sapogenin mixtures isolated from *Dioscorea* tubers[14].

Experimental

A slurry was prepared by mixing silica gel G, 30 g (Research Specialties Company, Richmond, Calif.) with distilled water, 60 ml. This was spread in a layer 0.5 mm thick on glass plates, 20 cm × 20 cm and after standing for 5 min the plates were dried at 100° for 30 min. The sapogenins were applied to the prepared plates as benzene solutions and after waiting for about 1 min for the spots to dry the plates were placed in one of three solvent systems: (1) chloroform-ethanol 95 % (95:5); (2) chloroform–acetone (3:1); and (3) ethyl acetate. Elution was by the supersaturated method of STAHL[15]. The procedure of BLUNDEN AND HARDMAN[14] afforded the test solutions: the tubers of *Dioscorea belizensis* Lundell, *D. sylvatica* Ecklon, and *D. villosa* Linn. were disintegrated, fermented, acid hydrolysed and the insoluble material was extracted with petroleum ether. Removal of the solvent gave a residue of sapogenin, 40 μg of which in benzene solution was used to spot the plate from a micropipette. Development of the plate occupied 60–90 min for both the chloroform–ethanol 95 % and the chloroform–acetone systems and 40–60 min for the ethyl acetate system. After development the chromatograms were dried for 2 min, sprayed with antimony trichloride in concentrated hydrochloric acid and heated at 90° for 10 min to enable the full colour of the spots to develop[16].

Results

The chromatographic pictures obtained from the sapogenin test solutions from all three *Dioscorea* species were very similar, irrespective of which solvent system was used. The pictures from *D. sylvatica* and *D. villosa* were identical and all these fractions were detected in the extract from *D. belizensis*. However, in addition to these

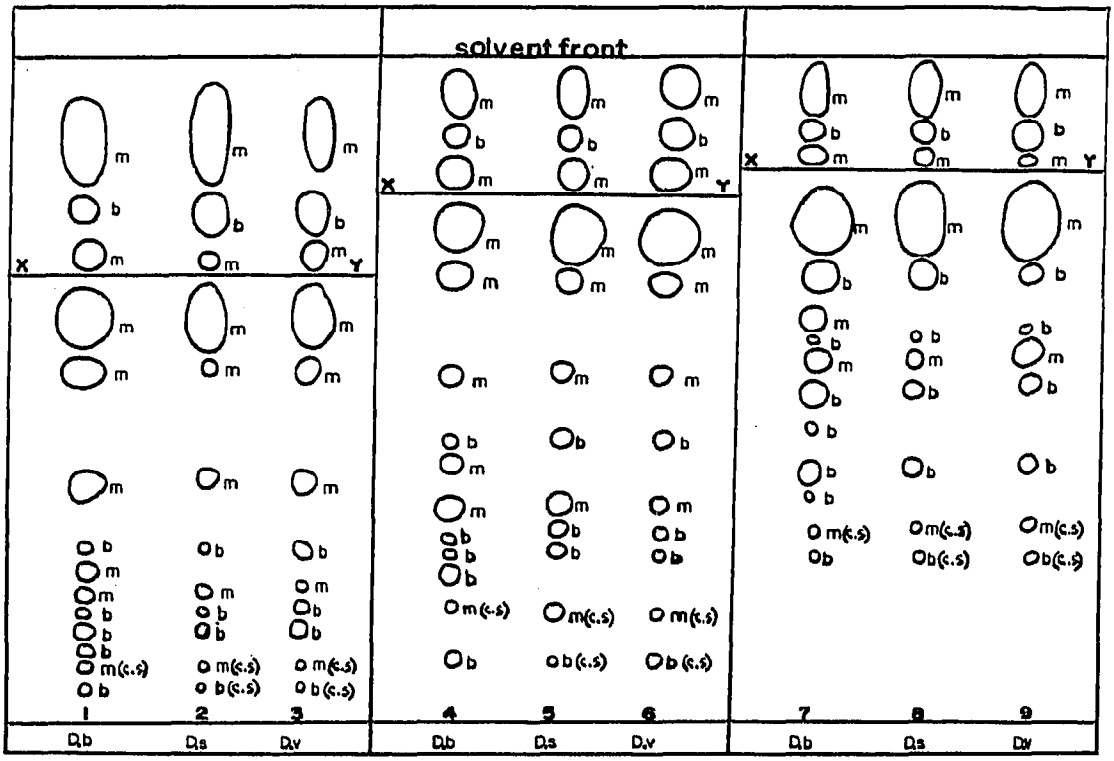

Fig. 1. Separation of steroidal sapogenins from *Dioscorea* species. D.b. = *D. belizensis*; D.s. = *D. sylvatica*; D.v. = *D. villosa*. Solvent systems: for 1, 2 and 3, chloroform–ethanol 95% (95:5); for 4, 5 and 6, chloroform–acetone (3:1); for 7, 8 and 9, ethyl acetate. Colour produced after spraying plates with antimony trichloride in concentrated hydrochloric acid and heating: m = mauve-purple, b = blue. Spots above the line XY were those detected from the first fractions of chloroform eluate from the sapogenin mixture after passage through an alumina column. Spots detected from concentrated solutions only = c.s.

compounds three others were detected from *D. belizensis*, but were not noticed in the chromatograms from the other two species (Fig. 1).

The spots with the highest R_F values were not clear using this concentration. These sapogenins were separated from the others by passage through an alumina column using chloroform as the eluting solvent and collecting the first fractions. Three spots were detected from these fractions from all three *Dioscorea* species (Fig. 1).

Samples of pure sapogenins were run alongside the plant extracts (Fig. 2). The reference compounds used were diosgenin (22α-spirost-5-en-3β-ol), kryptogenin (25α-cholest-5-en-3β,26-diol-16,22-dione), yamogenin (22β-spirost-5-en-3β-ol), botogenin (22α-spirost-5-en-3β-ol-12-one) and pennogenin (22α-spirost-5-en-3β,17α-diol). Of these the botogenin sample when chromatographed separated out into five different spots and could not be used as a reference. The reference compounds were in addition mixed with the tuber extracts from the three species of *Dioscorea* and chromatographed. The kryptogenin separated out from the spots produced by the plant extracts showing it to be absent from these extracts, but the diosgenin and pennogenin did not, indicating strongly their presence in the plant extracts. Diosgenin and its isomer yamogenin, were not clearly separated by any of the solvent systems. Diosgenin was the predominant sapogenin from all three plants when assayed by the standard procedure[14], and in tubers over two years old the following percentages

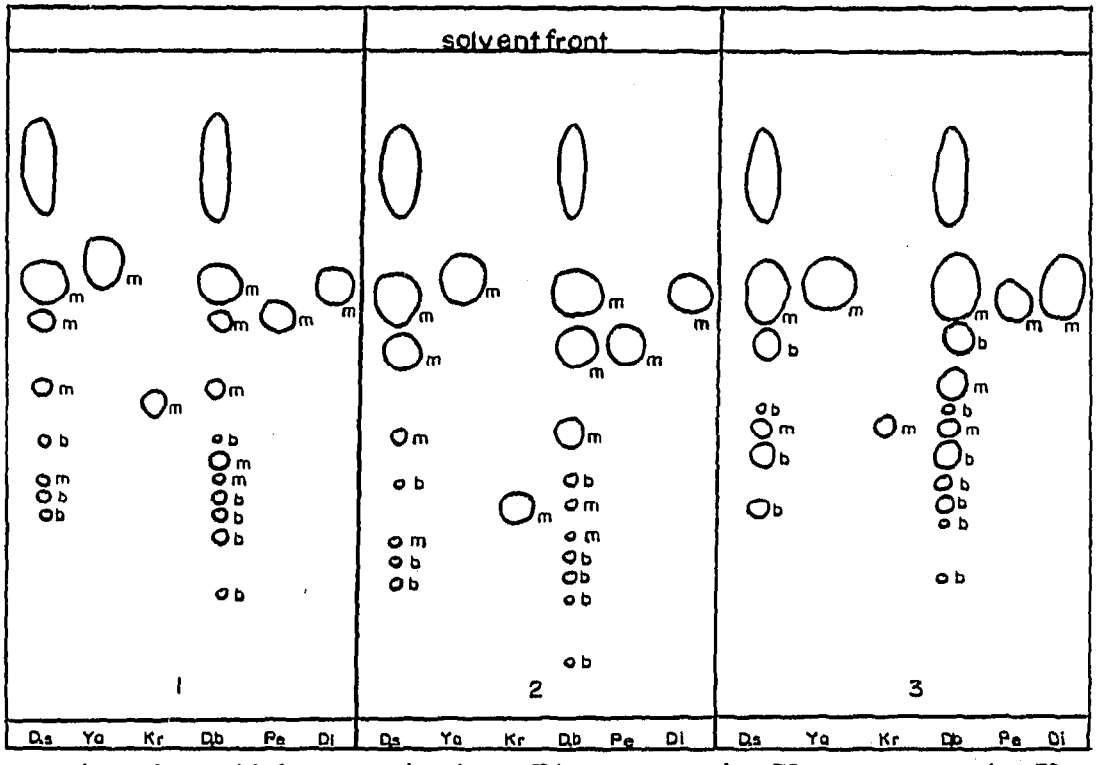

Fig. 2. Separation of steroidal sapogenins from *Dioscorea* species. Ya = yamogenin; Kr = kryptogenin; Pe = pennogenin; Di = diosgenin; D.b. = *D. belizensis* sapogenin mixture; D.s. = *D. sylvatica* sapogenin mixture. (Same spots also produced by *D. villosa*). Solvent systems: for 1, chloroform–ethanol 95% (95:5); for 2, chloroform–acetone (3:1); for 3, ethyl acetate. Colour produced after spraying plates with antimony trichloride in concentrated hydrochloric acid and heating: m = mauve-purple, b = blue.

were obtained, calculated on a moisture free basis: *D. villosa* 0.7% to 1.1%, *D. belizensis* 0.6% to 2.6%, *D. sylvatica* 4% to 6%.

Discussion

The saponin-containing *Dioscorea* tubers used in this study were selected both for their widely different geographical sources and for their representation of three different morphological types: the underground dwarf, rhizome-like organ of *D. villosa* Linn., indigenous to the eastern and central United States[17]; the underground large elongated tuber of *D. belizensis* Lundell, indigenous to British Honduras[18], and thirdly the large circular and plate-like tuber of *D. sylvatica* Ecklon, found in the surface of the soil of subtropical South Africa[19].

The use of all three developing systems proved worthwhile. For example, with the two chloroform systems the mauve-purple spots produced by diosgenin and pennogenin were separated from the extracts of all three *Dioscorea* species, but these two compounds were not separated using the ethyl acetate solvent system. However, with the latter system a blue spot was seen below the combined diosgenin–pennogenin spot; using the two other systems this blue spot was not separated from that of pennogenin. The presence of the two compounds was noticed after spraying, as the blue colour developed first and was then masked by the mauve-purple colour of the pennogenin on heating. Furthermore, the ethyl acetate system separated out more completely the blue spots of low R_F present only in the *D. belizensis* extract.

MARKER AND LOPEZ[20] demonstrated that the ethanolic acid or alkali treatment of the saponins from *D. mexicana* resulted in the formation of several sapogenins which were not naturally occurring in the plant as the glycoside. The large number of spots detected on the chromatograms in this present study probably include these products as well as any arising from disintegration and fermentation of the tuber. Diosgenin is the predominant sapogenin obtained from all three plants. Pennogenin was detected in all three plant extracts. It was shown by MARKER[20] AND LOPEZ that it could arise as a breakdown product. Kryptogenin was readily isolated from the Mexican species of *Dioscorea* studied by MARKER *et al.*[21], but they considered it to be a breakdown product formed during the isolation of the sapogenins[20]. Apparently it was not formed during our assay procedure, which avoids the use of boiling ethanolic hydrochloric acid, for kryptogenin was not detected in the sapogenin mixture from the Central American species *D. belizensis* nor from the other two species.

Detection of the other compounds present was impossible due to lack of reference compounds.

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