151. *The Saponin of* Sarcostemma australe, *R.Br.*

By JOHN W. CORNFORTH and J. CAMPBELL EARL.

The saponin of *Sarcostemma australe*, R.Br., is found to be a glucoside of an aglycone containing benzoyl and cinnamoyl ester groups. The ultimate hydrolysis product (*sarcostin*) has the composition $C_{21}H_{34}O_6,H_2O$ and is a well-crystallised substance, giving a *triacetate* on acetylation.

Sarcostemma australe, R.Br., is a latex-bearing plant growing in the arid parts of Australia. It was submitted to a preliminary chemical examination by Smith (*J. Proc. Roy. Soc. N.S.W.*, 1922, **56**, 183) and its reputed toxic properties were investigated by Gilruth and Murnane (*J. Counc. Sci. Ind. Res., Australia*, 1931, **4**, 225). The chemical constituents of the plant were examined in greater detail by Earl and Doherty (*ibid.*, 1937, **10**, 1). When an evaporated alcoholic extract of the plant was treated with ether, a residue was left which had saponin-like properties, and saponification of the ether-soluble material yielded a mixture of α- and β-amyrins. The saponin is now being investigated as part of a programme of study of the saponins and allied constituents of Australian plants.

The saponin differs from most saponins in being freely soluble in many organic solvents, so that partition methods may be used for its purification. The crude material originally isolated had the property of being precipitated as a syrup from its aqueous solutions on heating. The principal fraction from the systematic separation had the same property, but a further process of treatment removed it. No crystalline substance could be isolated, and all the fractions gave an emerald-green colour in the Liebermann–Burchard reaction.

Hydrolysis of the saponin was effected either with 0·75% methyl-alcoholic hydrogen chloride in a sealed tube at 100° or by boiling at atmospheric pressure with dilute aqueous alcoholic hydrochloric acid. In each case a brownish, ether-soluble resin was obtained. By the first method crystalline α-methylglucoside was isolated also, and the water-soluble products from the second method readily gave phenylglucosazone.

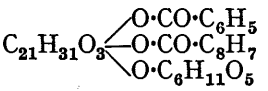
The resinous aglycone gave the same Liebermann–Burchard reaction as the saponin, but could not be obtained crystalline. It was decomposed by boiling with alcoholic potash to give a neutral genin and a mixture of acids identified as benzoic and cinnamic acids. The genin crystallised well and gave the characteristic green colour in the Liebermann–Burchard reaction. Its composition and properties do not correspond with those of any substance previously described, and the name *sarcostin* is therefore proposed for it.

As usually obtained, sarcostin contains water of crystallisation, which it loses on drying in a vacuum over phosphoric oxide at 100°. Its composition and molecular weight correspond to the formula $C_{21}H_{34}O_6,H_2O$ and this is confirmed by analysis of the anhydrous substance. On acetylation it yields a triacetate, $C_{27}H_{40}O_9$, from which the original sarcostin is regenerated on saponification. Both sarcostin and acetyl sarcostin were indifferent to carbonyl reagents.

The saponin was evidently a mixture of glucosides, the resinous aglycone being apparently a benzoyl cinnamoyl sarcostin, or a mixture of benzoyl and cinnamoyl sarcostins. The solubility of the saponin mixture in organic solvents and the fact that glucose and not a disaccharide was obtained on hydrolysis with very dilute acid, made it probable that the constituents of the mixture contained only one sugar residue per molecule. If the difference between the constituents of the mixture rested simply in the number and nature of acyl groups present, all the constituents should yield the same sarcostin glucoside on alkaline saponification. The saponin mixture was accordingly treated with alcoholic potash, and the neutral product acetylated. The resulting acetate gave on analysis results agreeing closely with the calculated values for *hexa-acetyl sarcostin monoglucoside*.

Quantitative saponifications carried out on the saponin mixture indicated that the average number of acyl groups per molecule lay between one and two. Some of the saponin fractions prepared by partition methods gave higher values, corresponding to two acyl groups. The saponin as a whole appeared therefore to consist mainly of a diacyl sarcostin monoglucoside, accompanied by less fully acylated glucosides. The quantity of glucose liberated on hydrolysis of the saponin confirmed this view.

Another point of interest was whether or no, in the case of a diacylated sarcostin glucoside, a benzoyl and a cinnamoyl group were attached to the same molecule. The substance chosen to determine this point was the resinous aglycone. This was submitted to chromatographic adsorption on aluminium oxide, whereby a main fraction was obtained of which the composition, saponification value and molecular weight approached fairly closely to those of a diacyl sarcostin. That this fraction was an individual substance was indicated by the results of a further chromatographic adsorption, which did not reveal the presence of more than one zone, and did not change the properties of the product. On saponification the purified substance yielded a mixture of benzoic and cinnamic acids in equimolecular proportion, just as did the crude aglycone. (A fraction of approximately the same composition was obtained from the crude aglycone by another method, and gave the same result on saponification.) It would appear therefore that the chief constituent of the aglycone is a *monobenzoyl monocinnamoyl sarcostin*, and that the principal component of the saponin mixture may be formulated in the annexed way.



An apparent anomaly in the relation between saponin and aglycone lay in the fact that, whereas the aglycone consisted almost entirely of diacylated sarcostin, the saponin contained some lower-acylated sarcostin glucosides, as the saponification values show. The explanation probably lies in the fact that sarcostin is sensitive to acids, and its acyl derivatives are less sensitive. Thus sarcostin glucoside, and possibly its monoacyl derivatives, would be transformed under the conditions of hydrolysis into insoluble tarry products. Support to this view is given by the fact that at least 10% of black tarry material was invariably produced during acid hydrolysis of the saponin.

Of biogenetic interest is the fact that some of the partly purified saponin and aglycone fractions responded weakly to the Legal nitroprusside test. It would seem that a small

proportion of a strophanthin-like glycoside is present in the mixture. At least one member of the natural order *Asclepiadaceæ*, to which *Sarcostemma australe* belongs, has been found to contain a cardiac glycoside, *viz.*, *Periploca græca*.

Work is now in progress on the fine structure of sarcostin, the results of which it is hoped shortly to communicate.

Experimental.

Note on Analytical Results.—All saponification and acetyl values given here were estimated by heating the substance with alcoholic alkali and back-titrating the excess with standard acid. The molecular weight determinations were all made by the Sucharda–Bobranski ebullioscopic method.

In the combustion of amorphous substances, the carbon values were invariably too low, and the hydrogen too high. The slightly hygroscopic nature of many of the resinous substances described below, and the great tenacity with which all of them retained occluded impurities (especially water), made correct analytical figures exceedingly difficult to obtain.

Extraction of the Plant.—The method devised by Earl and Doherty (*loc. cit.*) is thorough, but needed modification for larger quantities of the plant. The ground dry stems were steeped in alcohol for 24 hours at room temperature. The solvent was then drawn off and evaporated under reduced pressure. The plant material was again steeped in alcohol, the distillate from the first evaporation being used for the purpose. After 12 hours the process of drawing off and evaporating the alcohol was repeated. The almost exhausted plant material was again kept in contact with the recovered solvent, which was then used without distillation to extract a fresh charge of plant. For a charge of 8 kg. of plant, about 50—60 l. of alcohol were required. The residue from evaporation of the solvent was a thick syrup.

Isolation of the Crude Saponin.—The syrup was kneaded with successive quantities of ether, until the residue became solid and broke up into small particles. After standing for a short time under ether, the solid was filtered off as rapidly as possible, and immediately washed by decantation with a further quantity of ether. If the solid at this stage was allowed to remain in contact with the atmosphere, it became sticky and difficult to manipulate. Finally the solid was heated to remove occluded solvent. The yield of crude saponin was about 2·5% of the weight of plant material. It formed a light brown, amorphous powder, easily soluble in water to a strongly frothing solution, which on heating deposited the saponin as a syrup.

Partition Experiments.—Two successive processes were used to free the saponin on the one hand from sugars and inorganic impurities, and on the other hand from such fatty material as might have escaped the treatment with ether.

(i) The crude saponin was dissolved in about five times its weight of chloroform, and the solution shaken to an emulsion with half its volume of water. Alcohol was now added until the mixture separated fairly rapidly into two layers after shaking. The lower layer was separated and again shaken with water and sufficient alcohol to avoid emulsification. Finally it was again separated and evaporated, the residue being heated on the water-bath until free from solvent. The material thus obtained amounted to 90% of the weight of crude saponin. The aqueous-alcoholic washings on evaporation gave a dark hygroscopic syrup, which strongly reduced Fehling's solution and gave a positive osazone test. The main product of the partition (fraction A) was a yellowish powder, soluble in many organic solvents, including alcohol, ethyl acetate, dioxan, chloroform and acetone. Its solution in water behaved on heating in the same way as that of the crude saponin, but did not reduce Fehling's solution, showing that sugars were now absent.

(ii) The above product was now dissolved in three times its weight of alcohol, and an equal volume of benzene added. An amount of water slightly less than that of the benzene was introduced, and the mixture shaken and allowed to settle. The two layers were separated, and each was washed with fresh quantities of the complementary solvent. Each layer was evaporated separately, 85% of the material being found in the aqueous-alcoholic layer. In order to remove the last traces of benzene-soluble substances, this product was boiled with successive quantities of benzene, allowed to cool, and the solvent decanted or if necessary centrifuged off. The residue was freed from benzene by heating under reduced pressure on the water-bath. It then formed a pale yellow powder, and was entirely free from ash (Found : C, 61·0; H, 7·5%). This product (fraction B) was used for most of the degradations reported below. Its solubilities resembled those of fraction A. The benzene and benzene–alcohol solutions from the above operations yielded on evaporation a product which still contained a saponin, but was insoluble in water (Found : C, 66·8; H, 8·5%).

Fraction B was dissolved in water and shaken with ethyl acetate to an emulsion. The emulsion was allowed to settle for several days; the lower layer was then separated, and shaken with fresh ethyl acetate. The combined ethyl acetate extracts gave on evaporation a product (fraction C) richer in carbon than fraction B (Found: C, 61·9; H, 7·7%), and only sparingly soluble in water. The extracted aqueous solution yielded a product which dissolved in water to a solution not coagulated by heat. The amount of fraction C obtained was nearly half the weight of fraction B used.

All the saponin fractions obtained by the above processes gave a deep green colour when their solutions in acetic anhydride were treated with a little sulphuric acid.

Saponification of the Saponin.—The saponin (fraction B; 25 g.) was boiled with N/2-alcoholic potash (250 ml.) for 3 hours. The solution was neutralised to phenolphthalein, and the alcohol removed in a current of steam. The aqueous solution was shaken cautiously with three portions of ether, then evaporated almost to dryness on the water-bath. The residue was extracted several times with warm absolute alcohol. On evaporation of the alcoholic solution a brown resin remained which gave a strongly frothing solution in water, and an olive-green Liebermann–Burchard reaction. On hydrolysis with warm, very dilute hydrochloric acid, sarcostin was formed. It was difficult to free the resin from the last traces of inorganic material, so for analytical purposes an acetyl derivative was prepared. The product (3 g.) was dissolved in anhydrous pyridine (20 ml.), and acetic anhydride (10 ml.) added. After standing overnight, the solution was poured into water and the creamy solid (4·2 g.) was collected, dried, and dissolved in hot benzene. The filtered solution was evaporated, leaving a brownish resin soluble in most organic solvents, but showing no tendency to crystallise. After drying at 100° in a vacuum it was analysed (Found: C, 57·6; H, 7·3; OAc, 44·3. *Sarcostin glucoside hexa-acetate*, $C_{39}H_{56}O_{17}$, requires C, 58·8; H, 7·1; OAc, 44·5%).

The saponification values of fractions B and C were found to be 108 and 146 respectively. The calculated value for a diacyl sarcostin monoglucoside is 144, and for a monoacyl sarcostin monoglucoside, 85.

Hydrolysis of the Saponin.—(i) *With methyl-alcoholic hydrogen chloride.* The saponin (fraction B; 5 g.) was dissolved in methanol (50 ml.) containing 0·75% of dry hydrogen chloride, and the solution heated at 100° for 12 hours in a sealed tube. The solution was freed from mineral acid by shaking with a little silver carbonate, filtered, and heated on the water-bath until the alcohol was removed, water being added from time to time to replace it. The whole was then shaken with ether, a quantity of black tarry material (0·5 g.) remaining undissolved. The aqueous layer was evaporated, leaving a dark syrup which presently crystallised. The crystals were pressed on a tile and recrystallised from alcohol (charcoal). After a second crystallisation lustrous needles were obtained, m. p. 164—165° alone or in admixture with authentic α-methylglucoside.

The orange ethereal extract was shaken with dilute sodium carbonate solution, which removed a small amount of resinous acidic material, then dried (sodium sulphate), and evaporated. The crude resinous aglycone which remained weighed 2·1 g.

(ii) *With aqueous-alcoholic hydrochloric acid.* The saponin (fraction B; 10 g.) was dissolved in 1 : 1 aqueous alcohol (300 ml.), and concentrated hydrochloric acid added until the solution contained about 0·75% of hydrogen chloride. The mixture was boiled for 6 hours, the alcohol then removed in a current of steam, and the aglycone worked up with ether as described above. The yield of crude aglycone was 4·5 g., and of insoluble tar about 1 g. From the aqueous solution after removal of ether-soluble material, phenyl-*d*-glucosazone was obtained in the usual way, and identified by comparison with a specimen prepared from pure glucose.

In this method of hydrolysis the concentration of acid was varied between 0·5% and 2·5%, and the time of heating between 6 and 14 hours, without altering the nature of the product obtained. It was observed, however, that higher acid concentration and longer time of heating diminished the yield of aglycone, more tar being formed.

Sugar Content of the Saponin.—The following was a typical experiment: The saponin (fraction B; 3·213 g.) was heated with a mixture of 5% sulphuric acid (50 ml.) and absolute alcohol (50 ml.) for 6 hours on the water-bath. The solution was poured into a basin and heated on the water-bath, with occasional addition of water, until all alcohol was expelled. The aqueous solution was shaken thrice with ether, warmed to expel dissolved ether, and treated with a slight excess of barium carbonate. The precipitate was filtered off and repeatedly extracted with small quantities of hot alcohol. The combined filtrate and washings were evaporated to small bulk and made up to 100 ml. The sugar content of this solution was estimated by titration with Fehling's solution (Found: glucose, 0·76 g., *i.e.*, 23·7%. Calc. for a diacyl

sarcostin monoglucoside, 23·1%; calc. for a monoacyl sarcostin monoglucoside, 27·2%). In other experiments the concentration of glucose was checked by polarimetric measurements, which were in close agreement with the titration values.

Attempted Purification of the Aglycone.—(i) The crude aglycone (2 g.) was dissolved in hot benzene, and light petroleum added until a permanent turbidity appeared. On standing for a short time at 0° a resin separated (0·6 g.). After drying at 100°, it was analysed (Found: C, 70·8, 70·9; H, 7·5, 7·5%).

(ii) The crude aglycone (4 g.) was extracted (Soxhlet) for several days with light petroleum (b. p. 60—80°). The light yellow product (2 g.) which separated gradually from the boiling solvent was collected and dried at 100° in a vacuum [Found: C, 71·0; H, 7·45%; *M* (in benzene), 605].

(iii) The crude aglycone (5 g.), dissolved in benzene–chloroform (3 : 1; 60 ml.), was poured on a column of activated alumina (Merck; *nach* Brockmann), and the chromatogram developed with benzene–chloroform (2 : 1). The main product (2·1 g.) was found in a broad, nearly colourless zone near the top of the column. A second zone immediately below yielded 0·8 g. of slightly darker product. The main product was again chromatographed, the same procedure being used. One broad zone was formed, with very small rings of darker material above and below it. The product recovered from the column was almost white. It was dried at 100° in a vacuum for analysis [Found: C, 71·1; H, 7·3; saponification value, 177; *M* (in benzene), 603. *Monobenzoyl monocinnamoyl sarcostin*, $C_{21}H_{32}O_4(O{\cdot}CO{\cdot}C_6H_5)(O{\cdot}CO{\cdot}C_8H_7)$, requires C, 72·0; H, 7·2%; saponification value, 182; *M*, 616].

Saponification of the Aglycone.—This was accomplished by heating the aglycone with an excess of alcoholic alkali (N/2—N/5) for 2 hours on the water-bath. Water was then added, and the alcohol removed in a current of steam. The alkaline solution was shaken with a little ether, and allowed to stand for a time. Crude sarcostin crystallised and after some hours was filtered off and washed with water. The filtrate was extracted with ether in a continuous extraction apparatus for several days; further quantities of crystalline sarcostin separated from the boiling ether. From 50 g. of crude aglycone, 13—14 g. of crystalline product were obtained, along with 7—8 g. of resinous ether-soluble material. The aglycone purified by chromatographic analysis gave no resinous material on saponification.

Properties of Sarcostin.—The substance crystallised from ethyl acetate in highly characteristic, colourless, triclinic prisms, m. p. 266—267° after preliminary sintering. The crystals contained water of hydration, and sometimes melted when heated quickly to 170°, solidifying once more when the water was expelled [Found for air-dried material: C, 62·8, 62·9; H, 8·9, 8·9; loss at 100° over phosphoric oxide in a vacuum, 4·9; *M* (in ethyl alcohol), 393, 394, 412. $C_{21}H_{34}O_6,H_2O$ requires C, 63·0; H, 9·0; H_2O, 4·5%; *M*, 400. Found for anhydrous material: C, 65·9; H, 9·0; *M* (in ethyl alcohol), 384, 370. Calc. for $C_{21}H_{34}O_6$: C, 66·0; H, 8·9%; *M*, 382]. *Sarcostin* is moderately easily soluble in alcohol and pyridine, slightly in water and ether and hot ethyl acetate, and sparingly in most other solvents. In concentrated hydrochloric acid it dissolves on shaking to a deep blue-violet solution, from which resinous material presently separates. Sarcostin was also found to be resinified by heating with dilute mineral acids.

Sarcostin Triacetate.—Sarcostin monohydrate (1·8 g.) was dissolved in dry pyridine (7 ml.) and acetic anhydride (2·5 ml.). After several hours, the solution was poured into water and extracted with ether. The ethereal layer was washed with dilute hydrochloric acid, sodium carbonate solution, and water, dried (sodium sulphate), and evaporated. The residue was a colourless resin. On saponification with alcoholic alkali pure sarcostin was regenerated. For analysis, the *triacetate* was dried at 100° in a vacuum [Found: C, 63·6; H, 8·2; OAc, 33·0; *M* (in benzene), 500. $C_{21}H_{31}O_3(O{\cdot}CO{\cdot}CH_3)_3$ requires C, 63·8; H, 7·9; OAc, 34·8%; *M*, 508].

Identification of Benzoic and Cinnamic Acids.—The alkaline solution from the saponification of the aglycone, after the ether extraction, was made acid to Congo-red and extracted with ether. The dried ethereal extract yielded on evaporation a crystalline product, m. p. 70—80°. Samples from various aglycone fractions gave on titration with alkali equivalents ranging from 135 to 138 (Calc. for an equimolecular mixture of benzoic and cinnamic acids: equiv., 135). To identify the acids the crude product was esterified with alcoholic hydrogen chloride, and the product fractionally distilled under reduced pressure. Two fractions were obtained, which were separately saponified. The higher-boiling fraction yielded an acid which after two crystallisations from water melted at 133° alone or mixed with cinnamic acid. The acid from the lower-boiling fraction was also crystallised from water, and then melted at 118—120°. The m. p. was not depressed by benzoic acid, but the product evidently contained traces of cinnamic

acid, which were difficult to remove by crystallisation. The acid was therefore converted into the anilide, which was easily purified by crystallisation from alcohol and proved to be benzanilide by comparison with an authentic specimen.

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