267. *Sarcostin. Part I. A Preliminary Study of its Behaviour with Reagents.*

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Sarcostin is shown to contain a double bond, a $CH(OH)\cdot CH_3$ side chain, and two glycol groups open to attack by lead tetra-acetate. A number of unidentified crystalline products have also been isolated and some of these open the way to further study of the structure of the sarcostin molecule. Among the oxidation products is an acidic substance, $C_6H_8O_2$, which is probably 2-*methyl*-1 : 3-cyclo*pentanedione*, since it is easily oxidised further to succinic acid.

THE crystalline sapogenin, sarcostin, derived from the saponin of *Sarcostemma australe*, R.Br., has the composition $C_{21}H_{34}O_6$ (J., 1939, 737). It is sensitive towards acidic reagents. With cold concentrated hydrochloric acid it shows a characteristic colour change through red to violet-blue and yields an amorphous product of the probable composition $C_{21}H_{28}O_3$. For the degradative oxidation of sarcostin, lead tetra-acetate was selected as a suitable reagent: one molecular proportion was used very rapidly, a second less rapidly, and oxidation then continued slowly until 3—4 molecular proportions had been used. The products isolated were: (*a*) acetaldehyde, (*b*) a neutral *product*, $C_{21}H_{32}O_6$, m. p. 186—187°, (*c*) succinic acid, (*d*) another acidic substance, $C_6H_8O_2$, m. p. 210°, probably 2-*methyl*-1 : 3-cyclo*pentanedione*, and (*e*) a quantity of non-crystalline material.

Sarcostin triacetate uses only one molecular proportion of lead tetra-acetate and yields as principal product a crystalline *substance*, $C_{27}H_{40}O_{10}$, having ketonic properties. It can be oxidised further with potassium permanganate to a *substance*, $C_{23}H_{36}O_6$ or $C_{19}H_{30}O_5$. Benzoyl cinnamoyl sarcostin, the aglucone derived from the saponin by careful acid hydrolysis, behaves similarly with lead tetra-acetate, one molecular proportion only being used.

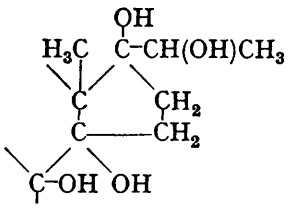
Hydrogenation of sarcostin in the presence of the Adams catalyst leads to *dihydrosarcostin*, $C_{21}H_{36}O_6$. Oxidation of dihydrosarcostin with lead tetra-acetate uses a little over two molecular proportions of the reagent and gives (*a*) acetaldehyde, (*b*) a neutral *product*, $C_{19}H_{28}O_5$, m. p. 194—195°, and (*c*) 2-methyl-1 : 3-*cyclo*pentanedione in much larger yield than from sarcostin itself. Dihydrosarcostin, like sarcostin, yields a *triacetate*.

Dehydrogenation of sarcostin with selenium gave a product which seemed to be Diels' hydrocarbon, but the quantity of material so far available has been insufficient for a positive identification to be made. Sarcostin condenses with acetone, the *product* being derived, apparently, from two molecules of sarcostin and one of acetone.

The experimental evidence so far accumulated is not sufficient for a complete determination of the molecular structure of sarcostin. Certain features of the structure have, however, been definitely established. The production of acetaldehyde during the oxidation of sarcostin and dihydrosarcostin indicates the presence of a $CH(OH)\cdot CH_3$ side chain. The fact that acetaldehyde is liberated during the early stages of the operation points to a 1 : 2 glycol structure, of which the secondary alcohol group of the side chain forms a part. The consumption of one molecule only of the oxidising agent when sarcostin triacetate is oxidised and the non-production of acetaldehyde in that case indicates that another glycol structure in the molecule is open to attack. The product of this oxidation is a neutral ketonic substance containing the same number of carbon atoms as sarcostin triacetate itself. A rational explanation of its formation is that both the hydroxyls forming the glycol group attacked are tertiary and have not been acetylated during the formation of sarcostin triacetate.

Apart from the rather slender dehydrogenation evidence, the fact that, when the side chain of sarcostin is removed, the remaining structure contains nineteen carbon atoms points to the skeleton of the molecule as a hydrogenated *cyclo*pentenophenanthrene structure with two angular methyl groups. The composition $C_{21}H_{34}O_6$, the absence of carbonyl, carboxyl and lactone groups, and the presence of one double bond are all in agreement with a molecular structure containing four rings. The identification of 2-

methyl-1 : 3-*cyclo*pentanedione as an oxidation product indicates the distribution of groups about the five-membered ring as in the annexed formula.



So far no product has been obtained from sarcostin which can be identified with any known sterol degradation product. Another relevant fact which must not be lost sight of is the close association of the saponin in the plant with the two amyrins (*J. Council Sci. Ind. Res. Aust.*, 1937, **10**, 1), which would lead to the expectation that the saponin would be of the triterpene class.

The progress of the investigation is reported at the present stage because it is temporarily interrupted by a shortage of material and the departure of one of the authors (J. W. C.) from Australia.

Experimental.

Action of Cold Concentrated Hydrochloric Acid on Sarcostin.—Sarcostin hydrate (2 g.), shaken with concentrated hydrochloric acid (40 ml.) at room temperature, dissolved rapidly to a red solution, which within 15 minutes became deep violet-blue. After 1½ hours the solution was placed in the ice-chest overnight and then poured into water. The precipitated solid was collected, dried, and extracted repeatedly with boiling ether until it was nearly completely dissolved. The ethereal solution, which showed a strong green fluorescence, was concentrated to 100 ml., decanted after 2 days from a small amount of dark resin, and evaporated to dryness. The product, after drying at 100°, was a yellowish-brown brittle resin (Found: C, 76·2; H, 8·2. Calc. for $C_{21}H_{28}O_3$: C, 76·8; H, 8·5%). It was not found possible to purify the product further.

Oxidation of Sarcostin by Lead Tetra-acetate.—Quantitative study. Sarcostin hydrate (0·1134 g.) was dissolved in purified glacial acetic acid (5 ml.) at 22°, and N/10-lead tetra-acetate (25 ml.) added. From time to time 2 ml. portions were run into sodium acetate–potassium iodide solution (Criegee, *Ber.*, 1931, **64**, 265), and the liberated iodine titrated with N/20-sodium thiosulphate. A parallel experiment with 0·1068 g. of sarcostin hydrate was also carried out. The results are tabulated below.

Expt. I.	Time, mins.	2	15	30	45	63	76	196	329	1181	1560
	$Pb(OAc)_4$, mols.	0·33	1·44	1·89	2·06	2·07	2·09	2·62	2·75	3·53	3·81
Expt. II.	Time, mins.	2	8	13	18	23	28	48	78	138	1043
	$Pb(OAc)_4$, mols.	0·18	0·76	1·18	1·50	1·63	1·72	1·97	2·01	2·53	3·37

Products of the oxidation. Several experiments were made. In general, sarcostin hydrate was dissolved in 10—100 times its weight of glacial acetic acid, treated with 2—3 mols. of lead tetra-acetate, and kept until the oxidising agent had been completely used. Acetaldehyde, a product of the reaction, was removed by passing carbon dioxide through the mixture and identified in the form of the *p*-nitrophenylhydrazone, m. p. and mixed m. p. 128—129°.

For the isolation of the other products, the reaction mixture was concentrated to small bulk at 40—50° under reduced pressure, poured into water, and filtered from the slight precipitate formed. Three products were then isolated.

(a) *Neutral product, m. p.* 186—187°. The solution was nearly neutralised with alkali, and extracted with ether in a continuous extractor. The ethereal solution was washed with dilute sodium carbonate solution and with water, dried over sodium sulphate, and evaporated to a syrup. On rubbing with acetone a crystalline *product* was obtained, which on recrystallisation from acetone formed flattened needles containing solvent of crystallisation. On heating, effervescence took place at 135—140°, after which the product resolidified and finally melted at 186—187°. The yield was 12 mg. from 1·5 g. of sarcostin hydrate. For analysis it was dried at 100° over phosphoric oxide under reduced pressure (Found: C, 65·8; H, 8·1. $C_{21}H_{32}O_6$ requires C, 66·3; H, 8·4%).

(b) *Succinic acid.* The aqueous solution from which the neutral products had been removed with ether was made acid to Congo-red and again extracted continuously with ether. The crystalline solid which separated from the ethereal solution during the extraction was recrystallised from ethyl acetate; it melted at 183—184° and was identified as succinic acid by comparison with authentic material. The yield was only 5 mg. from 1 g. of sarcostin hydrate, but oxidation of the neutralised aqueous solution, after extraction of the neutral products, with cold 1% potassium permanganate solution gave much larger yields of succinic acid (40 mg. from 1 g. of sarcostin hydrate).

(c) *Acidic substance, m. p.* 210°. In one experiment, in which the aqueous solution from the oxidation was extracted with ether without alkali first being added, the extract on slow evaporation deposited a crystalline solid (1·2 mg. from 1·16 g. of sarcostin hydrate). This crystallised from acetone in colourless leaflets, m. p. 208—210°. It was identical with the acidic product obtained in larger quantities from the oxidation of dihydrosarcostin (*q.v.*).

(d) *Other products.* In all the experiments the principal product of the oxidation was a yellow, neutral gum.

Sarcostin Triacetate and its Oxidation with Lead Tetra-acetate.—Sarcostin triacetate was previously reported as amorphous. It is, however, possible to crystallise it in well-formed prisms, m. p. 190°, by allowing a concentrated alcoholic solution to evaporate slowly in the air.

The oxidation of sarcostin triacetate (0·1288 g.) by lead tetra-acetate was studied quantitatively by the method already described. At the same time some of the aglucone (0·1386 g.) was oxidised similarly.

Acetate.	Time, mins.	5	15	25	50	120	180	235	
	$Pb(OAc)_4$, mols.	0·29	0·58	0·83	0·95	1·00	1·04	1·08	
Aglucone.	Time, mins.	5	18	28	40	72	120	180	1250
	$Pb(OAc)_4$, mol.	0·52	0·73	0·77	0·80	0·89	0·99	0·99	0·99

On a larger scale the oxidation was carried out in benzene solution. A solution of sarcostin triacetate (7 g.) in benzene (100 ml.) was stirred at room temperature, and lead tetra-acetate (6·1 g.; 1 mol.) added gradually. After 25 minutes the deposited lead acetate was filtered off and washed with benzene, the filtrate and washings being made up to 250 ml. with benzene. A 2 ml. portion of this solution required 2·18 ml. of N/10-sodium hydroxide for neutralisation (Calc. for 2 mols. of acetic acid, 2·20 ml.). Therefore no further acetylation had occurred during the oxidation.

After evaporation of the benzene under reduced pressure, the residue was taken up in alcohol. On standing, colourless crystals (1·4 g.) were deposited; a further quantity was obtained on concentrating the mother-liquor. On recrystallisation from alcohol the *product* (A) formed characteristic colourless prisms containing solvent of crystallisation. It melted at 90—110°, resolidified, and again melted at 164—165°. For analysis the substance was dried over phosphoric oxide at 100° under reduced pressure (Found: C, 61·7; H, 7·4; *M*, ebullioscopic in alcohol, 493. $C_{27}H_{40}O_{10}$ requires C, 61·8; H, 7·6%; *M*, 524).

Properties and Reactions of Product A.—The substance was unchanged by acetic anhydride in pyridine. It absorbed some hydrogen in the presence of the Adams catalyst, but no crystalline product could be isolated. Slow reaction occurred with Brady's reagent, an amorphous precipitate being formed. With boiling alcoholic alkali a deep red colour gradually developed; an amorphous precipitate was formed on acidification. With semicarbazide hydrochloride (165 mg.) and sodium acetate (165 mg.) in aqueous alcoholic solution, product A (150 mg.) gave, after boiling for 24 hours, a crystalline *semicarbazone*, which formed rhombic plates (15 mg.), m. p. 150—170° (decomp.), from alcohol (Found: C, 56·8; H, 8·0; N, 9·8. $C_{28}H_{43}O_{10}N_3$ requires C, 57·8; H, 7·5; N, 7·2%). It is apparent from the analytical figures that the product was not the pure monosemicarbazone, but the quantity available was insufficient for exhaustive purification.

Further Oxidation of Product A.—A solution of the substance (507 mg.) in benzene (10 ml.) was shaken with successive portions of cold 1% potassium permanganate solution (80 ml. in all) until decolorisation became slow. After filtration, the residue was extracted with water, and the solution acidified. A rancid-smelling, ether-soluble, acid material was obtained from which no crystalline product could be isolated. The residue was now extracted with hot alcohol, a crystalline *substance* (5 mg.) being obtained, m. p. 161—162°, depressed to 147° by product A (Found for material dried at 100° under reduced pressure: C, 67·4; H, 9·1. $C_{23}H_{36}O_6$ requires C, 67·6; H, 8·8%. $C_{19}H_{30}O_5$ requires C, 67·5; H, 8·8%).

Hydrogenation of Sarcostin.—A solution of sarcostin hydrate (2 g.) in glacial acetic acid (20 ml.) was shaken with platinum oxide (0·24 g.) in hydrogen (1 atm.); after 6 hours, 1 mol. was absorbed and reaction ceased. The solution was filtered, concentrated under reduced pressure, and poured into water. The precipitate, crystallised three times from alcohol–ethyl acetate, formed prisms, m. p. 245—246° (Found: C, 65·5; H, 9·7. $C_{21}H_{36}O_6$ requires C, 65·6; H, 9·4%). A further quantity of the material was recovered by continuous extraction of the aqueous filtrate with ether, the total yield being practically quantitative. *Dihydrosarcostin* gave a dull green colour with cold concentrated hydrochloric acid, and in the Liebermann reaction gave a yellow colour changing rapidly to green.

Dihydrosarcostin Triacetate.—Dihydrosarcostin (0·7 g.) was dissolved in pyridine (7 ml.), and acetic anhydride (1·5 g.) added. After 3 days the liquid was evaporated under reduced pressure, and the residue crystallised three times from hot alcohol. The *product* (0·25 g.) melted at 246—247° (Found: C, 63·1; H, 7·8; OAc, 34·3. $C_{27}H_{42}O_9$ requires C, 63·5; H, 8·2; 3OAc, 34·7%).

Oxidation of Dihydrosarcostin and its Acetate.—The oxidation was carried out quantitatively with lead tetra-acetate in the usual way.

Dihydrosarcostin (40·2 mg.).								
Time, mins.	12	20	31	46	63	117	189	1281
$Pb(OAc)_4$, mols.	0·36	0·61	0·78	0·94	0·95	1·21	1·51	2·28
Dihydrosarcostin acetate (39·5 mg.).								
Time, mins.	5	32	70	285				
$Pb(OAc)_4$, mols.	0·6	0·9	1·2	1·2				

For the isolation of the oxidation products, dihydrosarcostin (0·5 g.) was dissolved in glacial acetic acid (50 ml.), and lead tetra-acetate (1·1 g.; 2 mols.) added. After standing overnight, a 5 ml. portion of the solution was withdrawn, diluted with water, and distilled into *p*-nitrophenylhydrazine sulphate solution; acetaldehyde-*p*-nitrophenylhydrazone separated and was identified by comparison with an authentic specimen. The bulk of the reaction mixture was evaporated under reduced pressure, and the residue mixed with water. After 2 hours at 0° the crystalline material (150 mg.) was filtered off and recrystallised from water containing a little alcohol. The crystalline *substance* (B) contained water of crystallisation, which was expelled with effervescence at 110—115°, the anhydrous substance melting at 194—195° (Found: C, 67·8; H, 8·0; *M*, ebullioscopic in alcohol, 363. $C_{19}H_{28}O_5$ requires C, 67·9; H, 8·3%; *M*, 336).

The product showed no aldehydic reactions and could not be hydrogenated in the presence of the Adams catalyst (PtO_2). It was not further oxidised by lead tetra-acetate at room temperature.

The filtrate from product B was neutralised and extracted with ether, some gummy material being removed. It was then acidified with hydrochloric acid and extracted continuously with ether. A crystalline acidic substance (50 mg.) was obtained, which after recrystallisation from water melted at 210° and sublimed on prolonged heating at 200°. Its aqueous solution gave a violet colour with ferric chloride and decolorised permanganate in the cold (Found for material crystallised from water: C, 63·5; H, 7·3. Found for sublimed material: C, 63·5; H, 7·1; equiv., by titration, 112; *M*, by Barger's method in methanol solution, 112, 118. $C_6H_8O_2$ requires C, 64·3; H, 7·1%; equiv. and *M*, 112). The substance resembles in properties Eskola's 2-phenyl-1 : 3-*cyclo*pentanedione (*Chem. Abstr.*, 1938, **32**, 3359), which is acidic and gives colours with ferric chloride. The analytical figures and general properties indicate that the substance is probably 2-*methyl*-1 : 3-cyclo*pentanedione*, and confirmation was obtained by its oxidation to succinic acid by potassium permanganate. 6 Mg. were dissolved in 25 ml. of water, and a solution of 17 mg. of potassium permanganate in 5 ml. of water added. The resulting solution, which was only faintly pink, was boiled for a few minutes with a little carbon, filtered, and evaporated to dryness. The residue was treated with a few drops of hydrochloric acid and again evaporated. The solid material was extracted with ether and after evaporation of the ether a semi-crystalline syrup (4 mg.) remained. After pressing on a porous tile the crystalline portion melted at 182°, and when mixed with pure succinic acid at 184·5°. The rest of the material was mixed with ammonia, evaporated to dryness, and heated strongly; evidence of pyrrole formation was then obtained by the pine splinter reaction, confirming the identification of the substance as succinic acid.

Dihydrosarcostin acetate on oxidation gave a product which could not be crystallised, but which, like the corresponding product (A) from sarcostin acetate, gave a red colour on boiling with alcoholic alkali.

Dehydrogenation of Sarcostin.—Sarcostin hydrate (3·6 g.) and selenium (5 g.) were intimately mixed and heated rapidly to 280—290°. After 15 minutes the temperature was raised to 320—340° and maintained in that range for 20 hours except on two occasions when a rise to 360° was allowed to take place. After cooling, the product was extracted (Soxhlet) with ether. The oil remaining after removal of the solvent was distilled at 0·5 mm. pressure and collected between 160° and 170°. A trinitrobenzene complex was formed and recrystallised five times from alcohol, the m. p. then being 142° (the complex from the Diels hydrocarbon melts at 147°). Exhaustion of the material prevented further purification.

Condensation of Sarcostin with Acetone.—Sarcostin hydrate (0·5 g.) was dissolved in purified

acetone (15 ml.) containing 0·9% of hydrogen chloride. After a day the mixture was neutralised to phenolphthalein by the addition of N/10-alkali and poured into water. The *product* was extracted with ether and crystallised twice from benzene; it formed colourless needles, m. p. 225—256° (Found: C, 66·6, 66·8, 66·8; H, 8·8, 8·8, 9·1. $C_{45}H_{70}O_{12}$ requires C, 67·3; H, 8·7%). When a benzene solution of the product was kept in contact with dilute sulphuric acid, large and perfectly formed crystals of sarcostin appeared gradually at the interface.

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