942. *Some Reactions of Sarverogenin.*

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Sarverogenin, a cardiac aglycone occurring in several species of *Strophanthus*, has been degraded by ozonolysis and periodic acid oxidation. Some properties of the degradation products are reported.

Sarverogenin was first isolated by von Euw, Katz, Schmutz, and Reichstein (Festschrift Prof. Paul Casparis, Zürich, 1949, p. 178; cf. Buzas, von Euw, and Reichstein, *Helv. Chim. Acta*, 1950, **33**, 465) from the seeds of certain samples of *Strophanthus sarmentosus* P.DC., in which it occurs in combination with the sugar D-sarmentose as the highly cardioactive glycoside sarveroside. The aglycone is of common occurrence in *Strophanthus* species but has not been reported from any other genus.

Buzas *et al.* (*loc. cit.*) have shown that sarverogenin, to which they ascribe the formula $C_{23}H_{32}O_7$, gives a crystalline dibenzoate, and a positive reaction in the Legal test, indicative of the presence of an αβ-unsaturated butenolide grouping, such as occurs in other cardiac aglycones. Sarverogenin shows the strong ultra-violet absorption at about 218 mμ characteristic of this grouping, and a weak secondary maximum at 277·5 mμ, indicating the presence of a carbonyl group, although the substance does not react with the usual carbonyl reagents. These authors oxidised sarverogenin by chromic acid to a yellow substance, sarverogenone, apparently identical with " substance no. 782 " from the seed of *S. sarmentosus*.

The present author has obtained sarverogenin from the seeds of *S. amboënsis*, *S. courmontii*, *S. sarmentosus*, and *S. intermedius*, and found it identical in melting point and colour reactions with a specimen kindly provided by Professor T. Reichstein. However, analyses agree with the formula $C_{23}H_{30}O_7$ rather than with the $C_{23}H_{32}O_7$, but a final decision on this is postponed.

Of the seven oxygen atoms, two are accounted for in the unsaturated lactone ring, one as a carbonyl group, and two as hydroxyl groups. Active-hydrogen determination (Zerewitinoff) yields slightly more than three molecular proportions of methane, which, in consideration of the small amount always liberated by the unsaturated lactone grouping (Paist, Blout, Uhle, and Elderfield, *J. Org. Chem.*, 1941, **6**, 273), indicates the presence of a third hydroxyl group which is either tertiary or sterically hindered. There is as yet no evidence of the nature of the seventh oxygen atom.

Sarverogenin, unlike other cardiac aglycones, is not affected by refluxing with sulphuric acid (up to 5% v/v) in methanol, whereas sarmentogenin, for example, is converted into an anhydro-compound under these conditions (Callow and Taylor, *J.*, 1951, 2299). This property is useful in obtaining sarverogenin from difficultly hydrolysable glycosides containing 2-hydroxy-sugars linked directly to the genin nucleus; thus panstroside (sarverogenin digitaloside) is readily hydrolysed by acid of this concentration. Use of stronger acid gives lower recovery of sarverogenin and coloured substances, but even after 0·5 hour's refluxing with 10% (v/v) sulphuric acid in methanol about half of the original material can be recovered.

In alkali sarverogenin is extremely labile, a solution in alcoholic potash becoming rapidly yellow, but no crystalline material has been isolated from the product.

Sarverogenin readily consumes one mol. of periodic acid in aqueous dioxan or methanol, producing a compound, $C_{23}H_{30}O_8$, which has an ultra-violet absorption spectrum indistinguishable from that of sarverogenin itself, indicating that the lactone ring and a carbonyl group are still present. This compound is not extracted from chloroform by sodium hydrogen carbonate solution, though it is extracted with sodium carbonate and recovered after acidification, nor does it react with ethereal diazomethane or the usual carbonyl reagents. Acetic anhydride in pyridine gave only an amorphous acetate.

Further oxidation with chromic acid in acetic acid gives a good yield of a substance $C_{23}H_{26}O_8$ which still has the characteristic ultra-violet absorption spectrum, and is insoluble in sodium hydrogen carbonate solution and unaffected by ethereal diazomethane; it does not yield an acetate.

Sarverogenin dibenzoate is not oxidised by chromic or periodic acid, showing that the oxidation of sarverogenin by the latter reagent involves one of the acylatable hydroxyl groups. Since oxidation of a glycol does not increase the number of oxygen atoms in the molecule, the system oxidised by periodic acid must involve the carbonyl function, and, since carbon is not lost in the oxidation, the sarverogenin must be a secondary ketol.

Brink and Wallis (*J. Biol. Chem.*, 1946, **162**, 667) showed that periodic acid oxidised 3α : 12β-dihydroxy-11-ketocholanic acid to an aldehydo-acid with pronounced steric hindrance of the aldehydo- and the carboxyl-group, and it is possible that a similar steroid ring-C ketol may be involved in the present case.

Ozonolysis of sarverogenin diacetate gives an amorphous product which, after hydrolysis with potassium hydrogen carbonate and oxidation with periodic acid as described by Meyer and Reichstein (*Helv. Chim. Acta*, 1947, **30**, 1508) for the degradation of digitoxigenin, gives a carboxylic acid, $C_{24}H_{32}O_9$. The methyl ester, prepared with diazomethane, was

unchanged by phosphorus oxychloride and pyridine containing a trace of water at 110°, and although recovery decreased at higher temperatures no other crystalline material was obtained.

The ester was also recovered unchanged after attempted hydrogenation over Adams's catalyst and after treatment with chromic acid in acetic acid. Boiling methanolic sulphuric acid gave an amorphous substance, which however was not unsaturated to tetranitromethane and showed only ketonic absorption in the ultra-violet region. With alkali the ester, like sarverogenin itself, was labile, rapidly being converted into a yellow amorphous material; Wolff–Kishner reduction gave only an amorphous product.

The diacetate of the acid reacts with hydrazine in methanol with the loss of one of the acetyl groups to give a monoacetate acid, $C_{22}H_{30}O_8$, which is oxidised by periodic acid, showing that it is the acetyl group vicinal to the carbonyl group which has been eliminated. Since 3β : 11α-diacetoxy-14β-hydroxyetianic acid is recovered unchanged after treatment with hydrazine under the same conditions, the ready loss of the acetyl group appears to be due to the influence of the carbonyl function.

Experimental

M. p.s were determined on the Kofler block, and rotations in a 4-dm. tube.

Sarverogenin.—The richest source of sarverogenin so far found was in the seeds of an unidentified *Strophanthus* species obtained from Angola, probably *S. intermedius*, kindly provided by Dr. G. Taylor of the British Museum, Natural History. The minced seeds (218 g.) were extracted (Soxhlet) with methanol for 24 hours, at the end of which the residue was completely tasteless and was discarded. The extract was evaporated to about 200 c.c., extracted with light petroleum (2 × 400 c.c.), and diluted with 0·2N-sulphuric acid (250 c.c.). After extraction with a small amount of chloroform to remove the remainder of the oil and colouring matter, the solution was refluxed for 0·5 hour, cooled, and extracted with chloroform (2 × 250 c.c.). Evaporation of the chloroform left a crystalline residue (6·2 g.) which after two crystallisations from methanol furnished pure sarverogenin (4·85 g.), m. p. 232—234°, $[\alpha]_D^{19}$ +48·5° (*c*, 0·16 in MeOH), $\lambda_{max.}$ 218 (log ε 4·2) and 277·5 (log ε 1·83) (Found: C, 65·8, 65·8; H, 6·9, 7·0; active H, 0·78, 0·81. Calc. for $C_{23}H_{30}O_7$: C, 66·0; H, 7·2; 3 active H, 0·71%). Sarverogenin can crystallise in various forms with different m. p.s, but all of these go over to the form of m. p. 232—234° on recrystallisation from methanol. With 80% sulphuric acid it gave an immediate intense orange-red colour, which changed into a pure deep blue.

The dibenzoate, prepared by the method of Buzas, von Euw, and Reichstein (*Helv. Chim. Acta*, 1950, **33**, 465), and chromatographed on neutralised alumina, crystallised from methanol, in which it was only slightly soluble, as a crystalline powder, m. p. 189° (Found: C, 70·6; H, 6·1. Calc. for $C_{37}H_{38}O_8$: C, 70·9; H, 6·1%). With 80% sulphuric acid it slowly developed a carmine colour which eventually became greenish-blue. The colour reaction was much fainter than that given by sarverogenin under the same conditions, and the colours when compared side by side were distinct.

Action of Periodic Acid on Sarverogenin.—Sarverogenin (78·5 mg.) in dioxan (5 c.c.) was treated with a standard solution (1 c.c.) of periodic acid. Next morning, estimation of the periodic acid in this and in a control solution with sodium thiosulphate showed that the sarverogenin had consumed an amount of periodic acid equal to 3·0 c.c. of 0·1N-thiosulphate, which is equivalent to 0·82 mole of periodic acid per mole of sarverogenin.

Then, sarverogenin (1 g.) in dioxan (100 c.c.) was treated with a solution of periodic acid (1 g.) in water (5 c.c.). After being kept overnight the solution was diluted with water and concentrated *in vacuo* to remove the greater part of the dioxan. The crystals which separated were combined with a further amount obtained by extracting the mother-liquor with chloroform. After three crystallisations from methanol a *substance* (600 mg.) was obtained as prisms, m. p. 290—292°, $[\alpha]_D^{20}$ +5·3° (*c*, 0·18 in chloroform), $\lambda_{max.}$ 217 (log ε 4·13) and 275 (log ε 1·83) (Found, on samples dried at room temp.: C, 63·7, 63·4; H, 6·9, 6·9; after drying at 200° *in vacuo*: C, 63·9; H, 7·3; loss, 0·5%. $C_{23}H_{30}O_8$ requires C, 63·6; H, 6·9%). A small amount of sarverogenin could be recovered by careful working of the mother-liquors

The substance gave a strongly positive Legal test, but with 80% sulphuric acid gave only a pale yellow colour. It was recovered unchanged after treatment with semicarbazide or hydroxylamine in pyridine, and after 1 hour's refluxing in methanolic N-sulphuric acid. It gave no colour reaction with ferric chloride.

Oxidation of the Above Substance with Chromic Acid.—The substance (250 mg.) in acetic acid (5 c.c.) was treated with chromic acid (200 mg.) in water (2 c.c.). Next morning, the solution, which still contained unchanged chromic acid, was diluted with chloroform and water, and the organic layer washed with water, dried, and evaporated. The residue crystallised rapidly after dissolution in a little methanol, and after recrystallisation from acetic acid the *substance* had m. p. 228—231° (Found: C, 63·8, 64·0; H, 6·1, 6·35. $C_{23}H_{26}O_8$ requires C, 64·2; H, 6·0%). It was insoluble in all common solvents; with sulphuric acid it gave a yellow colour; the Legal test was positive.

Ozonolysis of Sarverogenin Acetate.—Sarverogenin (1 g.) was dissolved by gentle warming in pyridine (5 c.c.) and acetic anhydride (5 c.c.) and the solution left overnight. After dilution with methanol (10 c.c.) the solvents were removed *in vacuo* and the residual foam (1·4 g.) chromatographed on neutral alumina (50 g.). The acetate was eluted by ether–chloroform. All fractions remained amorphous but had sensibly the same optical rotation, $[\alpha]_D^{20}$ +26·8° (in $CHCl_3$). The colour reaction with sulphuric acid was similar to that described for the benzoate.

It has been found that a very suitable form of neutralised alumina for use with cardiac glycosides and related compounds can be prepared by washing ordinary activated alumina (Peter Spence, Type "H") made up in a column, with methyl formate and then with methanol. After drying at 100° the alumina is ready for use and does not require reactivation. The author is indebted to Dr. P. A. Robins for suggesting this method.

The combined fractions (1·0 g.) from the chromatography were ozonised in ethyl acetate (100 c.c.) at −80° until the solution became bright blue, then warmed to room temperature. Zinc dust (1 g.) and acetic acid (5 c.c.) were added, and the solution was kept for 0·5 hour and then diluted with water. The organic layer was washed with sodium hydrogen carbonate, dried, and evaporated, and the residual foam (1·2 g.) hydrolysed overnight with potassium hydrogen carbonate (1 g.) in water (30 c.c.) and methanol (65 c.c.). The solution was then diluted with water and ethyl acetate, and the organic layer dried and evaporated. To the residue (1 g.) in dioxan (45 c.c.), periodic acid (1·8 g.) in water (13 c.c.) was added. Next morning water and a drop of dilute sulphuric acid were added and the dioxan was removed *in vacuo*. The residue was extracted with ethyl acetate, the acidic part of the extract taken into sodium carbonate and then after acidification back in to chloroform, and the chloroform layer dried and evaporated. After addition of methanol the *diacetoxy-acid* (500 mg.) crystallised, and after recrystallisation from methanol had m. p. 234—236°, $[\alpha]_D^{21}$ +38·0° (*c*, 0·1 in $CHCl_3$) (Found: C, 61·5, 61·8; H, 6·7, 6·9. $C_{24}H_{32}O_9$ requires, C, 62·1; H, 6·9%). The acid gave no colour with alcoholic ferric chloride.

The crude acid (from sarverogenin, 2 g., but without chromatography of the acetate) with ethereal diazomethane gave the *methyl* ester (1·15 g.) prisms (from methanol), m. p. 201—202°, $[\alpha]_D^{20}$ +40° (*c*, 0·33 in $CHCl_3$), $\lambda_{max.}$ 278·5 (log ε 1·78) (Found, on a sample dried at room temperature: OMe, 13·1, 13·4; OAc, 17·2; active H, 0·3. $C_{25}H_{34}O_9,CH_3{\cdot}OH$ requires 2OMe. 12·15; 2OAc, 16·9; 2 active H, 0·4. In a sample dried at 100° *in vacuo*: C, 62·7, 62·8; H, 7·1, 7·4; OMe, 7·2. $C_{25}H_{34}O_9$ requires C, 62·8; H, 7·1; 1OMe, 6·5%). The ester gave no colour with alcoholic ferric chloride.

Action of Hydrazine on the Diacetoxy-acid.—The acid (250 mg.) and hydrazine hydrate (0·5 c.c.) were kept in methanol (5 c.c.) overnight at room temperature, and the solution was diluted with water, acidified with dilute sulphuric acid, and extracted with chloroform. Evaporation gave the *monoacetoxy-acid* which crystallised from methanol in needles (200 mg.), m. p. 253—255°, $[\alpha]_D^{19}$ +48·5° (*c*, 0·8 in $CHCl_3$) (Found: C, 62·6, 62·7; H, 7·1, 7·0; OAc, 10·4; N, 0. $C_{22}H_{30}O_8$ requires C, 62·6; H, 7·1; 1OAc, 10·2%). Esterification with diazomethane gave a gum from which a few crystals, m. p. 145—150°, were obtained, but it has not been possible to obtain this ester satisfactorily crystalline.

The acid product (200 mg.) absorbed 0·95 mol. of periodic acid but no crystalline product was isolated.

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