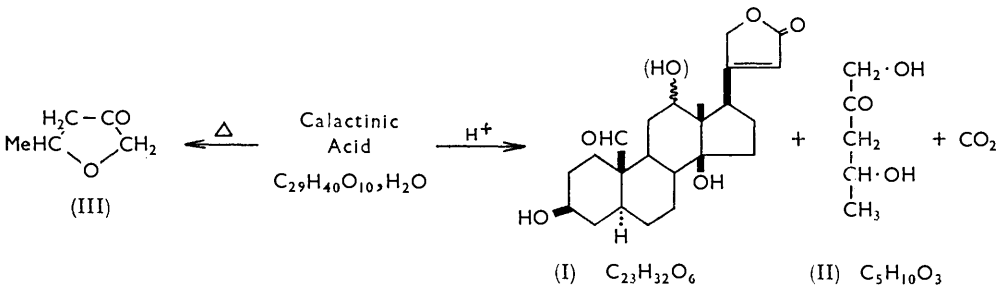
347. *Cardenolides. Part V.*[1] *The Constitution of Calactinic Acid.*

By D. H. G. Crout, R. F. Curtis, and C. H. Hassall.

Degradative experiments lead to structure (IX) for calactinic acid.

It has been shown [2] that calactin, $C_{29}H_{40}O_9$, may be converted under mild conditions into calactinic acid which is, in turn, readily hydrolysed by acid to calotropagenin, $C_{23}H_{32}O_6$, and fragments derived from a C_6 residue. This reaction sequence made calotropagenin, the aglycone common to the six cardenolide " glycosides " of *Calotropis procera*,[3] readily available for investigations of structure. It also offered some prospect of obtaining more direct evidence of the nature of the unusual C_6 " glycoside " functions in this series of compounds; for, in spite of extensive and fruitful investigations on these " glycosides," the methods of pyrolysis and alkaline hydrolysis that have been used for cleavage could evidently modify the products of degradation. This made difficult the interpretation of the results, so that it has not hitherto been possible to assign unequivocal structures to these unusual " glycosides " or to account for their reactions in a satisfactory way. The studies on calactinic acid which will be described lead, we believe, to an unambiguous assignment of structure to this compound and, through this, to a satisfactory interpretation of the chemistry of the " glycosides " themselves.

Earlier investigations on calactinic acid [2] indicated the molecular formula $C_{29}H_{40}O_{10}$ and showed that acid hydrolysis, under mild conditions, produced calotropagenin * (I), carbon dioxide, and a C_5 fragment which could be isolated as a 2,4-dinitrophenylosazone; this has been identified as the optically active derivative of 4-hydroxy-2-oxopentanal.[1]



Pyrolysis of calactinic acid yields a volatile product which has been identified as (−)-tetrahydro-2-methyl-4-oxofuran (III) by comparison with synthetic material.[1] This compound is also formed as a very minor product of acid hydrolysis of calactinic acid. These results suggest that the C_5 fragment produced on hydrolysis is 1,4-dihydroxypentan-2-one (II), the location of the carbonyl group being indicated by its position in the compound (III) that could be derived from (II) by cyclisation. There is additional evidence for this formulation. When the hydrolysis mixture is oxidised with sodium metaperiodate, formaldehyde and β-hydroxybutyric acid are formed, and if the hydrolysis mixture is reduced with sodium borohydride, (4*R*)-pentane-1,2,4-triol ($[\alpha]_D^{22}$ $-12° \pm 2°$) is obtained. The *D*(or *R*)-configuration at position 4 follows from the fact that the optical rotation is

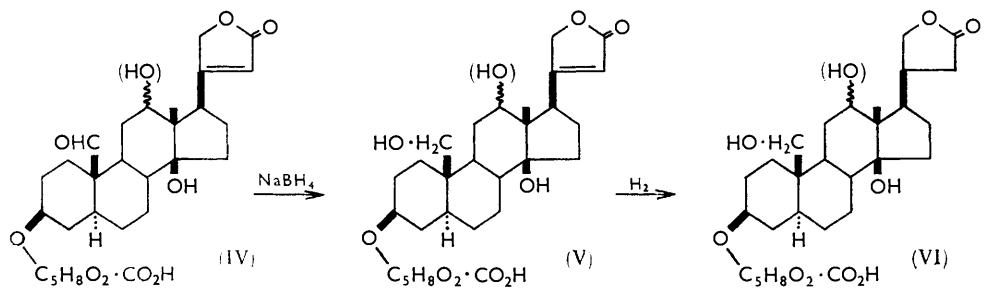
* The assignment of the hydroxyl group to the 12-position in calotropagenin is tentative. It is based on evidence which favours the 11- or 12-positions [2,4] and on unpublished evidence [5] that excludes the 11-position.

[1] Part IV, Curtis, Hassall, and Weatherston, *J.*, 1962, 4225.
[2] Hassall and Reyle, *J.*, 1959, 85.
[3] Hesse, Heuser, Hütz, and Reicheneder, *Annalen*, 1950, **566**, 130.
[4] Bharucha, Hesse, Jager, Weiss, and Reichstein, *Helv. Chim. Acta*, 1962, **45**, 93.
[5] Hassall and Roderick, unpublished results.

opposite in sign to that of (4*S*)-pentane-1,2,4-triol ($[\alpha]_D^{16}$ +15° ± 2°) which was prepared by hydroxylation of (+)-pent-1-en-4-ol.[1] (−)-Pent-1-en-4-ol has been correlated through (−)-α-hydroxybutyric acid [6] and (−)-lactic acid [7] with D (or *R*)-glyceraldehyde.[8,9]

The carbon dioxide released during hydrolysis is evidently derived from a carboxyl group in calactinic acid. The presence of this group in tetrahydrocalactinic acid, in which it will be shown that the 10-formyl and the 17-butenolide group have been reduced so as no longer to mask the carboxyl absorption in the infrared absorption spectrum, is clearly indicated by a band at 5·82 μ; in the case of the corresponding methyl ester, this band is no longer apparent but there is enhanced absorption at 5·69 μ. Calactinic acid is monobasic. The pK_a value, 4·0, is comparable with that of acids with oxygen groups in the α-position (glycollic acid, 3·8; lactic acid, 3·9) rather than simple carboxylic acids (acetic acid, 4·8; propionic acid, 4·9).

Attempts to achieve acid hydrolysis of calactinic acid or its methyl ester under such mild conditions that the carboxyl group remains attached to the C_5 unit, have been unsuccessful. It has been necessary, therefore, to make a more detailed study of calactinic acid itself in order to define the oxygen functions and to establish the point of attachment of the carboxyl group to the C_5 unit.



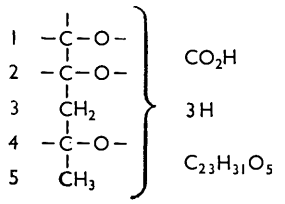
When dihydrocalactinic acid, the product of reduction of calactinic acid with sodium borohydride, was hydrolysed under the same conditions as calactinic acid, dihydrocalotropagenin, carbon dioxide, and the ketol (II) were formed. Catalytic hydrogenation of dihydrocalactinic acid led to an uptake of one mol. of hydrogen. The product, tetrahydrocalactinic acid, gave no Kedde reaction, indicating that the butenolide ring had been reduced; on acid hydrolysis it gave an uncharacterised steroid fraction, carbon dioxide, and the ketol (II). Evidently, these reduction processes have led to modification of the aglycone unit alone and may be represented by the sequence (IV) ⟶ (VI). Clearly the C_6 unit does not contain a group such as a carbonyl group, that is subject to such reduction processes.

Calactinic acid and dihydrocalactinic acid form a tri- and a tetra-acetate, respectively. It follows from the structures (IV) and (V) that the C_5 fragment must contain two acylable centres.

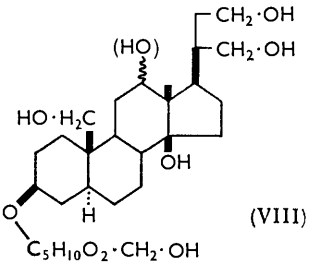
Neither calactinic acid nor dihydrocalactinic acid reacts with periodic acid. This, taken with the evidence on the behaviour of calactinic acid on reduction, precludes a partial structure (VII) for the " glycoside " in which calotropagenin is joined through the oxygen at position 4. Several points of evidence contribute to allocation of the carboxyl function in the partial structure (VII). Tetrahydrocalactinic acid may be reduced with lithium aluminium hydride to a compound with no carbonyl absorption in the infrared spectrum. Although this compound has not been obtained crystalline, analyses were in agreement with the formula $C_{29}H_{52}O_9$ and both the compound and its acetylation product gave

[6] Levene and Haller, *J. Biol. Chem.*, 1929, **81**, 425.
[7] Levene and Haller, *J. Biol. Chem.*, 1926, **67**, 329.
[8] Brewster, Hughes, Ingold, and Rao, *Nature*, 1950, **166**, 178.
[9] Cahn, Ingold, and Prelog, *Experientia*, 1956, **12**, 81.

single spots on paper chromatograms. It seems reasonable to assign the partial structure (VIII) to this polyol. There is certainly no feature in the reduced cardenolide portion of (VIII) that could react with sodium periodate. Nevertheless, the polyol takes up 1 mol. of sodium periodate and releases one equivalent of formaldehyde. This is attributed to fission involving the $-CH_2 \cdot OH$ group derived by reduction of the carboxyl group in calactinic acid. Although the polyol (VIII) was resistant to acid hydrolysis under conditions that were effective with calactinic acid, some cleavage was achieved with boron



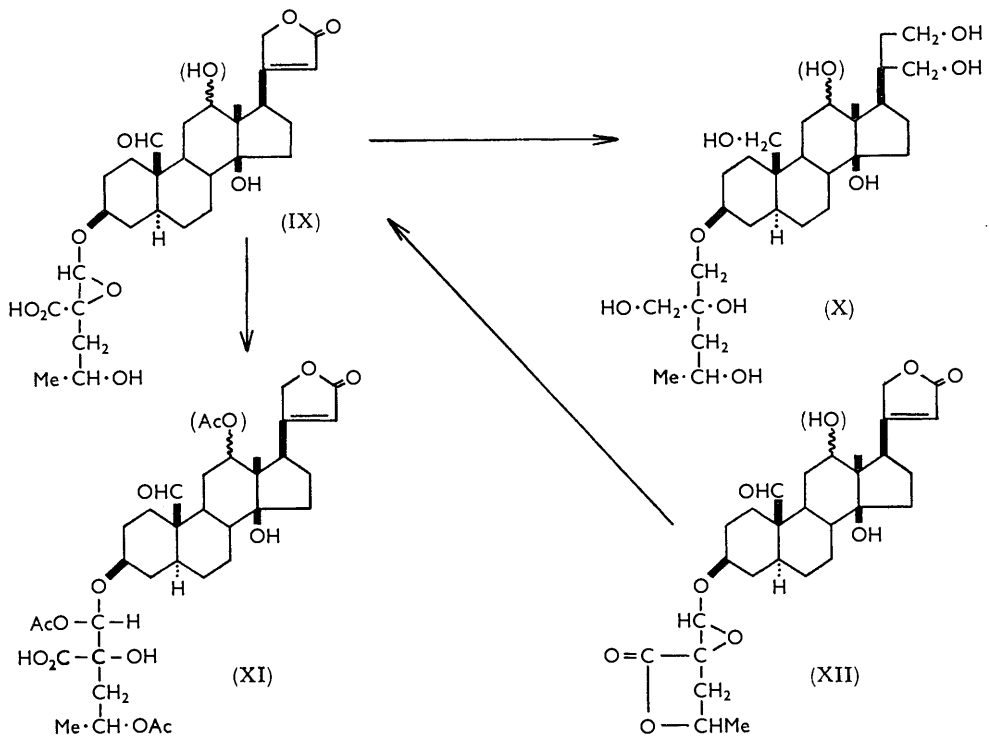
(VII)



(VIII)

trichloride, a reagent that has been used successfully for the cleavage of methyl ethers of monosaccharides.[10] The " glycosidic " fragment reacted with sodium periodate to give a single acidic product which was identified as β-hydroxybutyric acid.

The formation of formaldehyde when the polyol (VIII) is oxidised with sodium periodate indicates that the carboxyl group in the partial structure of calactinic acid (VII) must be



attached to one of the positions 1, 2, and 4 which bear oxygen. The formation of β-hydroxybutyric acid on periodate oxidation of the reduced glycosidic fragment limits the point of attachment to position 2. This, in turn, defines the point of attachment of the glycosidic

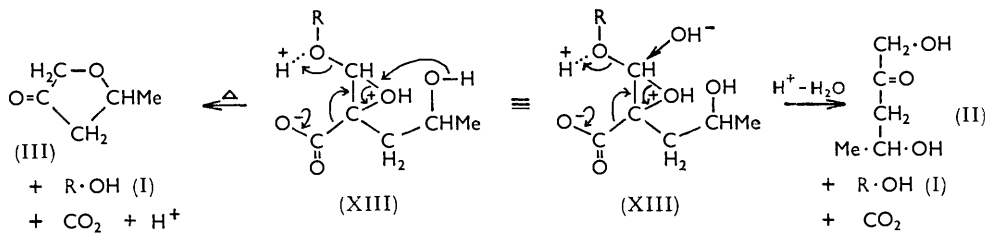
[10] Allen, Bonner, Bourne, and Saville, *Chem. and Ind.*, 1958, 630.

link as position 1, since position 4 has already been excluded and attachment to position 2 would not allow periodate cleavage of the polyol.

It only remains to account for one oxygen atom at position 2. This must be capable of acylation and of activating decarboxylation through acid hydrolysis or pyrolysis. It must be associated with either a double bond or a ring and must allow interpretation of the hydrolysis, pyrolysis, and reduction with lithium aluminium hydride.

These requirements are all met by the formulation of calactinic acid as the epoxide (IX). On this basis the product of reduction with lithium aluminium hydride is the polyol (X): the resistance of the epoxide ring in (IX) to catalytic hydrogenation finds analogy in other cases where one, or both, of the carbon atoms are tertiary.[11] Acylation follows the well-established pattern of reaction of nucleophilic reagents with epoxy-ethers[12] to yield the triacetyl derivative (XI).

The behaviour of calactinic acid on pyrolysis and acid hydrolysis is also explained in terms of the structure (IX). It is well known that glycidic acids are readily decarboxylated, particularly when there are α-alkyl substituents.[13] The products of pyrolysis or acid-catalysed hydrolysis are ketones. Detailed evidence of the mechanism of such reactions is lacking. However, it has been suggested for simple cases[14] that initial attack of the proton occurs on the epoxide ring to yield the conjugate acid:



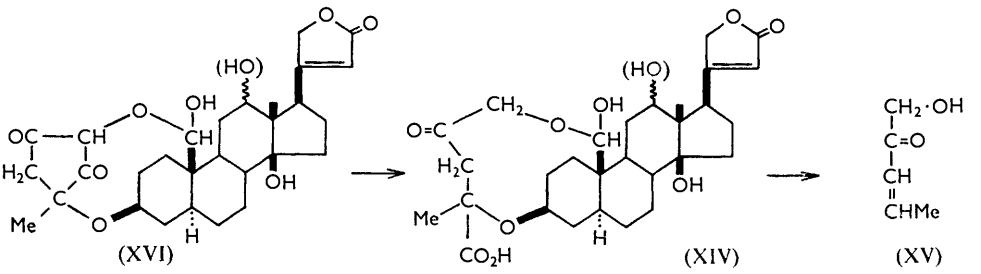
In this case such a conjugate acid, represented as (XIII), might very well be decarboxylated and give rise to the aglycone by a sequence of steps in which the formation of either (4*R*)-1,4-dihydroxypentan-2-one (II) or (—)-tetrahydro-2-methyl-4-oxofuran (III) was dependent on whether a hydroxyl ion or the hydroxyl group at position 4 was involved in the reaction.

In an earlier study[2] it was shown that treatment of calactin with silica gel gave a neutral compound, $C_{29}H_{40}O_9$, m. p. 305—307°, which was converted into calactinic acid by the action of alumina. This high-melting compound does not react with sodium periodate. Mild alkali in the presence of water or methanol gives calactinic acid or its methyl ester, respectively, while acid-catalysed hydrolysis gives the same products as from calactinic acid. This compound is evidently the lactone (XII) corresponding to calactinic acid.

Recently Hesse and Lettenbauer[15] have proposed the structure (XIV) for " boraxsaure," which we have shown to be identical with calactinic acid. The structure (XIV) was based largely on the assumption that 1-hydroxypent-3-en-2-one (XV) was the initial product of hydrolysis of calactinic aid and that the ketol (II) was derived from it by hydration. This assumption is no longer tenable.[1] Further, it was suggested that uscharidin should be formulated as (XVI) and that a compound with this structure could be converted into (XIV) by very mild alkali.

[11] Tarbell, *et al.*, *J. Amer. Chem. Soc.*, 1961, **83**, 3096.
[12] Parker and Isaacs, *Chem. Rev.*, 1959, **59**, 737.
[13] Dullaghan and Nord, *J. Org. Chem.*, 1953, **18**, 878; Morris and Lusth, *J. Amer. Chem. Soc.*, 1954, **76**, 1237; Morris and St. Lawrence, *ibid.*, 1955, **77**, 1692; Morris and Young, *ibid.*, p. 6678.
[14] Pritchard and Long, *J. Amer. Chem. Soc.*, 1956, **78**, 2663, 2667, 6008; 1957, **79**, 2367; Gould, " Mechanism and Structure in Organic Chemistry," H. Holt and Co. Inc., New York, 1960, p. 291.
[15] Hesse and Lettenbauer, *Annalen*, 1959, **623**, 142.

The structure (XIV) does not account for some important properties of calactinic acid and its derivatives. In particular, it is excluded by the evidence of periodate oxidation of the reduced forms of the acid. Dihydrocalactinic acid, the product of reduction of calactinic acid by sodium borohydride, yields dihydrocalotropagenin on hydrolysis. As dihydrocalactinic acid has no formyl group at position 10 of the steroid nucleus, the possibility of



semiacetal formation no longer exists and the reduced acid should react readily with periodic acid. However, there is no uptake during 21 hours. Similarly, the formation of β-hydroxybutyric acid by periodate cleavage of the product of hydrolysis of the polyol, $C_{29}H_{52}O_9$, cannot be explained in terms of the structure (XIV).

Experimental

Ultraviolet absorption spectra were determined for ethanol solutions with a Unicam S.P. 500 spectrophotometer and an Optica CF4 recording spectrophotometer. Infrared absorption spectra were measured for potassium bromide discs with a Grubb-Parsons GS 2A spectrometer and a Perkin-Elmer " Infracord " instrument. Potentiometric titrations were carried out in aqueous methanol (4%) by using a Radiometer titrator TTT 1a with 0·2N-sodium hydroxide.

Alumina for chromatography was Spence's grade H, washed with dilute nitric acid, water, and methanol and activated for 12 hr. at 120°. In all experiments involving extraction of butenolide components from aqueous solution, at least six extractions were necessary and these were continued until the aqueous phase gave no Kedde reaction.

Quantitative periodate titrations were carried out by the acid–iodine technique.[16] Periodic acid or sodium metaperiodate solution used was 0·01—0·03M in all cases control experiments were carried out simultaneously. Oxidations were made in the dark at room temperature. The glycoside (10—20 mg.) was used in ~0·005M-solution and after the oxidation the excess of iodate and periodate were estimated by liberation of iodine from acidified potassium iodide and the iodine was titrated in the usual way.

Paper chromatography was on Whatman No. 1 paper using the following systems: System no. (1), paper impregnated with formamide irrigated with chloroform saturated with formamide; [15] (2), paper impregnated with octan-1-ol and irrigated with water saturated with octan-1-ol; [15] (3) butan-1-ol–acetic acid–water (4 : 1 : 5); (4) butan-1-ol–hydrochloric acid–water (10 : 2 : 3·6); (5) butan-1-ol–toluene–acetic acid–water (2 : 2 : 1 : 5); (6) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3); (7) butan-1-ol saturated with 1·5N-ammonia; [17] (8) propan-2-ol–1·5N-ammonium carbonate in 3 : 1 (v/v) H_2O–aqueous ammonia (*d* 0·88); [18] (9) as no. 8 but with propan-1-ol; and (10) paper impregnated with formamide irrigated with benzene–cyclohexane (2 : 1) saturated with formamide. Spray reagents used were: (A) Kedde reagent prepared by mixing immediately before use equal volumes of 3,5-dinitrobenzoic acid (2%) in methanol and N-sodium hydroxide; (B) after drying of the paper at 110°, 10% phosphomolybdic acid in methanol, papers being then heated at 110° for 2 min.; [19] (C) periodate spray [sprayed with aqueous ammonia (*d* 0·88) and then, after drying, with aqueous 0·01M-sodium metaperiodate; after 4 min. sprayed with soluble starch (3%), potassium iodide solution (0·8%), and boric acid (0·9%) in saturated aqueous sodium tetraborate (30%); positive reactions were

[16] Dyer, *Methods Biochem. Analysis*, 1956, *III*, 111.
[17] Lederer and Reid, *Biochem. J.*, 1951, **50**, 60.
[18] Kalbe, *Z. physiol. Chem.*, 1954, **297**, 19.
[19] Kritchevsky and Kirk, *Arch. Biochem. Biophys.*, 1952, **35**, 346.

white spots on a blue background]; [20] (D) triphenyltetrazolium chloride (2%) mixed before use (v/v) with N-sodium hydroxide; (E) 0·1M-silver nitrate in 5M-aqueous ammonia and papers heated to 110°; [21] (F) spray E with the addition of sodium hydroxide until concentration was 2N; [21] and (G) Bromocresol Purple (0·04% w/v) in ethanol–formalin (5 : 1), with pH adjusted to 5·0 by 0·1N-sodium hydroxide.[17]

Calactinic Acid (IX).—We have already described [2] the small-scale preparation of calactinic acid from calactin isolated from *Calotropis procera*. The following procedure is a considerable modification which produces calactinic acid directly from the plant material.

Dried and powdered root bark (1·8 kg.) was suspended in light petroleum (b. p. 60—80°) (6 l.) in an inverted bell jar fitted with a drain tap and a large pad of glass wool as filter. The slurry was stirred and set aside for 1 hr. while the solvent drained. This process was repeated with three further 2-l. portions of solvent. The combined extracts were distilled, to give a viscous gum (81 g.) which was not further investigated.

The residual root bark was allowed to dry overnight by drawing a current of air through the jar and then suspended in chloroform–ethanol (4 : 1; 6·5 l.). The slurry was stirred for 45 min., allowed to settle for 15 min., and then drained. This process was repeated with four further portions (3·5 l.) of the same solvent mixture and the combined extracts were distilled under reduced pressure to yield a sticky brown residue (26 g.).

The brown residue (75 g.) was dissolved in chloroform (100 ml.) and reprecipitated by pouring the solution with rapid stirring into pentane (1 l.). The pentane was decanted, the residue redissolved in chloroform (1·5 l.), and the process repeated in pentane (1·5 l.). One further treatment gave a brown friable powder (26·3 g.). This was dissolved in acetone (400 ml.) and water (100 ml.) and shaken with mercuric chloride (12 g.) and calcium carbonate (3 g.). Further amounts of calcium carbonate (each 3 g.) were added after 3, 19, and 51 hr., the mixture being shaken occasionally. After 70 hr. the solution was filtered, then cooled in ice–water, and the excess of mercury was precipitated with hydrogen sulphide. The solution was again filtered and acetone was removed under reduced pressure, to give a brown resin free from sulphur-containing compounds.

This brown resin (46 g.) was dissolved in chloroform (1 l.), filtered, and treated with Spence's alumina (grade H; 600 g.). The mixture, after being shaken for 10 min. and set aside for 67 hr., was introduced on a column of activated alumina (500 g.) and eluted with chloroform (2 l.), methanol (2 l.), chloroform–methanol–water (5 : 6 : 2; 3·5 l.), and 5% aqueous sodium hydrogen carbonate (2·2 l.).

The sodium hydrogen carbonate eluate was acidified and shaken with chloroform–ethanol (4 : 1) until the Raymond reaction was negative. Evaporation of the combined extracts gave needles, m. p. 170—172° (2·64 g.). One crystallisation from ethanol gave calactinic acid (IX), m. p. 169—172°, $[\alpha]_D^{22}$ −37·3° ± 2° (*c* 0·62 in pyridine), $\lambda_{max.}$ 218, 307—309 mμ (log ε 4·19, 1·55), pK_a 4·0 (Found: C, 61·2; H, 7·6; *C*-Me, 5·2, 5·4. Calc. for $C_{29}H_{40}O_{10},H_2O$: C, 61·5; H, 7·5; 2*C*-Me, 5·3%).

A sample of " boraxsaure " kindly supplied by Professor G. Hesse showed m. p. 169—172° (mixed m. p. with calactinic acid, 169—172°), $[\alpha]_D^{22}$ −39·6° ± 2° (*c* 0·625 in pyridine), and had an identical infrared absorption spectrum.

The R_F of both acids (system 2, spray A) was 0·80. The iodoform test was strongly positive. There was no reaction with 0·21M-sodium metaperiodate in methanol–water (5 : 1) during 42 hr.

The *methyl ester* was obtained when calactinic acid in methanol was treated with an excess of diazomethane in ether at 0°. Crystallisation from ethanol gave rectangular plates, m. p. 212—221°, $[\alpha]_D^{22}$ −20·8° ± 2° [*c* 0·58 in ethanol–chloroform (4 : 1)], $\lambda_{max.}$ 218, 302—308 mμ (log ε 4·20, 1·68) (Found: C, 64·2; H, 7·6; O, 28·7; OMe, 5·4. $C_{30}H_{42}O_{10}$ requires C, 64·0; H, 7·6; O, 28·4; 1OMe, 5·5%).

The *triacetate* (XI) was produced when calactinic acid (60 mg.) in pyridine (2 ml.) was treated with acetic anhydride (1 ml.) at room temperature for 48 hrs. Crystallisation from aqueous methanol gave needles (34 mg.), m. p. 170—171° (Found: C, 60·9; H, 6·9; OAc, 17·2. $C_{35}H_{46}O_{13},H_2O$ requires C, 60·7; H, 7·0; 3OAc, 18·6%). In an earlier study [2] it was suggested that this was a diacetate. The carbon and hydrogen values are ambiguous but repeated acetyl determinations indicated a triacetate.

Hydrolysis of Calactinic Acid: Periodate Oxidation of Water-soluble Fraction.—The aqueous

[20] Metzenberg and Mitchell, *J. Amer. Chem. Soc.*, 1954, **76**, 4187.
[21] Partridge, *Nature*, 1946, **158**, 270.

solution obtained when calactinic acid was hydrolysed by dilute acid [1] was separated from calotropagenin, neutralised with silver carbonate, and after filtration treated with an excess of hydrogen sulphide. The filtered solution was concentrated at 35° under reduced pressure, to give a liquid which was shown by chromatography in systems 3, 4, and 5 (spray D) to contain one reducing component (R_F 0·61, 0·84, and 0·53, respectively).

A similar experiment with calactinic acid (1·73 g.) gave an aqueous solution (350 ml.) which was treated with sodium metaperiodate (1·5 g.) in water (10 ml.). After 7 hr. in the dark, the excess of periodate was reduced with sulphur dioxide and the solution was treated with a 0·25% solution (600 ml.) of 2,4-dinitrophenylhydrazine in 2N-sulphuric acid. Precipitated solid was collected (235 mg.; m. p. 155—163°) and after chromatography on alumina in benzene crystallised from methanol to give formaldehyde 2,4-dinitrophenylhydrazone, m. p. and mixed m. p. 169—170°. The R_F values on paper chromatography in di-isopentyl ether–formamide [1] were identical.

Aqueous hydrolysate (80 ml.) from the hydrolysis of calactinic acid (320 mg.) was treated with sodium metaperiodate (363 mg.) in water (20 ml.) for 24 hr. in the dark. The excess of periodate was removed by ethylene glycol (100 mg.), and the product was extracted with peroxide-free ether for 24 hr. The ether extract was dried and distilled and the residue distilled at 60°/1 mm., to give a colourless oil (12·6 mg.). This oil and the ether extract showed only one acidic component identical with β-hydroxybutyric acid, R_F 0·11, 0·52, 0·44 on paper chromatography in systems 7, 8, and 9 (spray G). No crotonic acid (R_F 0·26, 0·64, 0·61 in the same systems) could be detected.

(4R)-*Pentane*-1,2,4-*triol from Calactinic Acid.*—Calactinic acid (1·94 g.) was hydrolysed with dilute sulphuric acid, and the aqueous portion was neutralised with barium carbonate. The precipitate was collected and the filtrate was taken to dryness at 35°. The residue was dissolved in methanol (30 ml.), filtered, and treated with sodium borohydride (145 mg.) in 1 : 1 aqueous methanol (12 ml.). After 1 hr. the reaction with alkaline triphenyltetrazolium chloride was negative and a further portion of sodium borohydride (50 mg.) was then added. After being kept for 23 hr. the solution was acidified with 2N-sulphuric acid (5 ml.), and methanol was removed at 30°. The residue was deionised by filtration through a mixed column of Amberlites I.R. 50 (4 g.) and I.R. 45 (4 g.), eluted with water (80 ml.), and evaporated at 25°. Decolorisation with charcoal (8 mg.) in ethanol followed by evaporation to dryness gave (4R)-*pentane*-1,2,4-*triol* (120 mg.) as a colourless oil, n_D^{23} 1·4660, $[\alpha]_D^{22}$ −12° ± 2° (*c* 5·8 in ethanol). Curtis *et al.*[1] give $[\alpha]_D^{16}$ +15° ± 2° for the (4*S*)-isomer. The infrared absorption spectrum was very similar to that of the authentic (4*S*)-isomer and showed no absorption between 5·0 and 6·0 μ. Paper chromatograms of both compounds in systems 5 and 6 (spray C) showed a single spot, with R_F 0·53, 0·61.

Periodate Oxidation of (4R)-*Pentane*-1,2,4-*triol.*—The foregoing triol (60 mg.) in water (30 ml.) was treated with periodic acid dihydrate (350 mg.) in the dark for 2 hr. The excess of periodate was removed with sulphur dioxide, and the solution was treated with 2,4-dinitrophenylhydrazine solution (as above) (200 ml.) and heated on the water-bath for 10 min. Next morning the precipitated red solid (91 mg.) was collected and chromatographed on activated alumina in dichloromethane to give crotonaldehyde 2,4-dinitrophenylhydrazone (64 mg.) as needles, m. p. and mixed m. p. 192—193·5°.

Dihydrocalactinic Acid (V).—Calactinic acid (1·04 g.) in 4 : 1 methanol–water (50 ml.) was treated with sodium borohydride (1·012 g.) in the same solvent mixture (60 ml.). After 5 hr. further sodium borohydride (315 mg.) was added, and then after a further 24 hr. the solution was acidified with 2N-sulphuric acid, and methanol was removed at 40°. A chloroform–methanol (4 : 1) extract of the aqueous solution was evaporated, to give *dihydrocalactinic acid* (V) as needles (962 mg.), m. p. 188—191°, $[\alpha]_D^{22}$ −56·2° ± 2° (*c* 0·68 in pyridine), $\lambda_{max.}$ 218 mμ (log ε 4·14), pK_a 4·0 (Found: C, 61·5; H, 8·3; O, 30·3. $C_{29}H_{42}O_{10},H_2O$ requires C, 61·3; H, 7·8; O, 30·9%). With periodic acid in methanol–water (5 : 1) there was no uptake of periodate after 21 hr.

The *methyl ester* prepared from the acid by treatment with an excess of ethereal diazomethane in methanol at 0° recrystallised from ethanol as an *ethanol solvate*, prisms, m. p. 204—220° (sintering 155—160°), $[\alpha]_D^{22}$ −28·4° ± 2° (*c* 0·6 in ethanol), $\lambda_{max.}$ 219 mμ (log ε 4·14) [Found (in material dried at room temperature under a high vacuum): C, 62·7; H, 8·3; loss in wt. at 120°, 8·8. $C_{30}H_{44}O_{10},C_2H_5{\cdot}OH$ requires C, 62·9; H, 8·3; EtOH, 7·55%. Found (in material dried at 100°): C, 64·0; H, 8·2; O, 28·4; OMe, 6·4. $C_{30}H_{44}O_{10}$ requires C, 63·8; H, 8·0; O, 28·3; 1OMe, 5·5%].

The same ester was obtained on reduction of methyl calactinate with sodium borohydride.

The *tetra-acetate* was obtained from the acid (81 mg.) in acetic anhydride (1 ml.) and pyridine (2 ml.) after 48 hr. at room temperature, as needles (48 mg.), m. p. 168—169° (from ethanol), $[\alpha]_D^{22}$ −54·4° ± 2° (*c* 0·58 in ethanol), $\lambda_{max.}$ 217 mμ, (log ε 4·15) (Found: C, 61·2; H, 7·4; OAc, 22·8, 23·4. $C_{37}H_{50}O_{14},0{\cdot}5H_2O$ requires C, 61·1; H, 7·1; 4OAc, 23·7%).

Hydrolysis of Dihydrocalactinic Acid.—The acid (987 mg.) was heated under reflux for 7 hr. in methanol (70 ml.), water (70 ml.), and 0·05N-hydrochloric acid (30 ml.) in a stream of nitrogen. Issuing gas was passed through 0·05N-barium hydroxide; titration indicated 0·75 mol. of carbon dioxide evolved.

Methanol was removed at 25° and the residue was extracted with chloroform–ethanol (4 : 1) which was dried and evaporated. The residue was triturated with ethyl acetate and filtered; insoluble material crystallised from ethanol to give unchanged dihydrocalactinic acid (66 mg.). Soluble material was purified by chromatography on activated alumina in ethyl acetate and elution with ethyl acetate–ethanol (7 : 3). The main fraction recrystallised, to give dihydrocalotropagenin (340 mg.) as plates (from ethanol–chloroform), m. p. and mixed m. p. 259—260°, with the same R_F values in system 1 (0·01) and in system 2 (0·57) (spray A) as authentic material.[2]

The aqueous residue from the extraction with chloroform–ethanol was neutralised with silver carbonate, filtered, treated with hydrogen sulphide, filtered again, evaporated to dryness at 35°, and dissolved in dry methanol (25 ml.). A portion (5 ml.) of this solution was warmed with an excess of 2,4-dinitrophenylhydrazine solution (as above) on the water-bath for 1 hr. The orange-red precipitate (43 mg.) was purified by chromatography on activated alumina, eluted with chloroform–ethyl acetate (99 : 1), and crystallised from ethyl acetate to give (+)-4-hydroxy-2-oxopentanal 2,4-dinitrophenylhydrazone, m. p. 250—252°, identical with authentic material.[1]

Two further portions of the methanolic solution (5 ml. each) were treated with sodium metaperiodate (200 mg.) in water (45 ml.), and the products worked up as described under calactinic acid, giving formaldehyde 2,4-dinitrophenylhydrazone (17 mg.) and β-hydroxybutyric acid (1·5 mg.).

The remaining methanolic solution was examined by paper chromatography. In systems 3, 4, and 5 developed with sprays, C, D, E, and F, only one component was detected; it was identical in R_F value with 1,4-dihydroxypentan-2-one (II) obtained by hydrolysis of calactinic acid.

Tetrahydrocalactinic Acid (VI).—Dihydrocalactinic acid (641 mg.) in methanol (400 ml.) was shaken under hydrogen with Adams catalyst (60 mg.) at room temperature and pressure. After 4 min. uptake of hydrogen ceased (23·9 ml.) and there was no further uptake during 10 hr. (calc. for 1 double bond, 24·2 ml.). The product was obtained as needles (637 mg.), m. p. 166—167°. Three crystallisations from ethanol gave *tetrahydrocalactinic acid*, m. p. 168—169°, $[\alpha]_D^{22}$ −43·7° ± 2° (*c* 0·61 in ethanol) (Found: C, 63·2; H, 8·6. $C_{29}H_{44}O_{10}$ requires C, 63·0; H, 8·0%), R_F (system 2, spray B), 0·76. The infrared absorption spectrum showed a peak at 5·82 μ (CO_2H group); the Raymond reaction was negative.

The *methyl ester*, prepared with diazomethane in the usual way, formed needles (from ethanol–ether), m. p. 251—254°, $[\alpha]_D^{22}$ −43·2° ± 2° (*c* 0·63 in ethanol), $\lambda_{max.}$ 208 mμ (log ε 2·3) (Found: C, 63·6; H, 8·3; O, 28·4; OMe 6·4. $C_{30}H_{46}O_{10}$ requires C, 63·6; H, 8·2; O, 28·2; 1OMe, 5·5%), R_F (system 1, spray B), 0·39, $\nu_{max.}$ 5·69 μ (CO_2Me).

Hydrolysis of Tetrahydrocalactinic Acid.—The acid (183 mg.) was heated under reflux for 2 hr. with methanol (10 ml.) and 0·1N-hydrochloric acid (20 ml.) in a stream of nitrogen. Carbon dioxide was evolved (0·85 mol.). The product was worked up as described for the hydrolysis of dihydrocalactinic acid, and the aqueous portion examined by paper chromatography in systems 3, 4, and 5. With spray D only one reducing component could be detected; it had R_F identical with that of 1,4-dihydroxypentan-2-one (II) from calactinic acid.

Polyol, $C_{29}H_{52}O_9$ (X).—Tetrahydrocalactinic acid (101 mg.) in a Soxhlet apparatus was extracted into a solution of lithium aluminium hydride (2·0 g.) in boiling tetrahydrofuran (120 ml.) over a period of 5 hr. After being kept overnight the excess of reagent was destroyed with ethyl acetate, the mixture was acidified, and volatile solvents were removed at 30°. The residue was extracted with chloroform–ethanol (4 : 1; 10 × 50 ml.). Evaporation gave a yellow gum which was introduced in the minimum volume of ethanol and ethyl acetate on to a column of deactivated alumina (9 g.). Material which was eluted with ethyl

acetate was rejected. Elution with ethyl acetate–ethanol (1 : 1) gave the *polyol* (X) (87·4 mg.) as a colourless foam which was dissolved in water and passed through a column of mixed Amberlites CG 120 and CG 400 (4 g.; 1 : 1) to remove inorganic material. Removal of solvent under reduced pressure gave the polyol as a colourless foam. It was also obtained as an amorphous powder by precipitation with ethyl acetate from a concentrated solution in methanol. The compound was extremely hygroscopic and retained water tenaciously. This led to analytical results that varied with the conditions of drying. It was not possible to obtain anhydrous material since drying at elevated temperatures led to decomposition [Found (for material dried at 120°): C, 60·0; H, 9·2. $C_{29}H_{52}O_9,2H_2O$ requires C, 60·0; H, 9·7%]. The infrared absorption spectrum showed no absorption in the carbonyl region and the material showed only one spot, R_F 0·03 on paper chromatography in system 1 (spray B). With 0·02M-sodium metaperiodate in dioxan–water (5 : 1) there was an uptake of 1·0 mol. of reagent in 48 hr.; in methanol–water (5 : 1), 0·93 mol. was taken up in 91 hr.

An acetyl derivative was prepared by treatment with acetic anhydride–pyridine solution in the usual way for 48 hr. Paper chromatograms (spray B) in systems 1 and 2 showed single spots, R_F 0·84 and 0·85, respectively.

Oxidation of the Polyol (X) *with Sodium Metaperiodate.*—The polyol (21·2 mg.) in ethanol (1 mol.) and water (2 ml.) was treated with sodium metaperiodate (16 mg.) in the dark for 22 hr. 0·01N-Sodium arsenite (11 ml.) was added and, after 50 min., water (10 ml.) and sodium acetate buffer (pH 4·6, 44 ml.). 0·4% Dimedone solution (16 ml.) was added and the mixture was set aside for 24 hr., then the precipitate was collected, (10·3 mg.; m. p. 186°) (Calc. for 1 mol. of formaldehyde, 11·5 mg.). Recrystallisation from aqueous ethanol gave the dimedone derivative of formaldehyde as needles (7 mg.), m. p. 189—191°. There was no depression of the m. p. with authentic material and the infrared absorption spectra were identical.

Cleavage of the Polyol (X) *with Boron Trichloride.*—The polyol (22 mg.) was treated with boron trichloride (8 g.) under anhydrous conditions at −70°. The mixture was kept at this temperature for 30 min. and then allowed to warm to room temperature. When the reagent had evaporated, methanol (2 ml.) was added and the solution was poured into water (20 ml.). After addition of an excess of silver carbonate, filtration, passage of hydrogen sulphide, and filtration again, the solution was evaporated to dryness at 30°. The residue was dissolved in chloroform–ethanol (4 : 1, 10 ml.) and shaken with water (5 ml.), which was re-extracted with chloroform–ethanol (4 : 1). The aqueous layer was then concentrated at 30° and the residue dissolved in methanol (2 ml.). Paper chromatography of this material in system 5 and development with spray C revealed only one component, R_F 0·37.

This methanolic solution was concentrated (0·2 ml.) and treated with sodium metaperiodate (10 mg.) in water (2 ml.) in the dark for 18 hr. The excess of periodate was removed with sulphur dioxide, water (10 ml.) was added, and the solution was extracted continuously with ether for 6 hr. The ethereal extract was dried and evaporated and the residue was examined by paper chromatography in systems 7 and 8 (spray G). Only single spots, R_F 0·10 and 0·52, respectively, corresponding to β-hydroxybutyric acid were obtained. There was no indication of crotonic acid. The residue of the ether extract gave no derivative of crotonaldehyde when treated with 2,4-dinitrophenylhydrazine. A similar result was obtained in the cleavage experiment when the polyol was replaced by its acetyl derivative.

Reactions of Calactinic Acid Lactone (XII).—(*a*) *With sodium metaperiodate.* The lactone, prepared from calactin by the action of activated silica gel,[2] had m. p. 305—307° (Found: C, 65·5; H, 7·7; O, 26·9. Calc. for $C_{29}H_{38}O_9$: C, 65·6; H, 7·2; O, 27·1%). It did not react with 0·0178M-sodium metaperiodate in methanol–chloroform–water (5 : 1 : 1) during 22 hr.

(*b*) *With sodium hydrogen carbonate.* The lactone (19 mg.) in chloroform–methanol–water (5 : 6 : 2) was allowed to react at room temperature for 42 hr. with sodium hydrogen carbonate (40 mg.). Separation of the products into water-soluble and chloroform–ethanol (9 : 1)-soluble fractions gave in the former fraction calactinic acid (1 mg.) and in the latter methyl calactinate (9 mg.), m. p. and mixed m. p. 210—220° (correct infrared spectrum).

(*c*) *Hydrolysis with sulphuric acid.* Heating the lactone (9 mg.) with N-sulphuric acid (2 ml.) and ethanol–water (9 : 1, 2 ml.) for 30 min. was followed by working up as in the case of hydrolysis of calactinic acid. Paper chromatograms of the products in system 5 (sprays C, E) and system 6 (sprays C, D, E) showed only one spot with R_F's corresponding to those of 1,4-dihydroxypentan-2-one (II). In system 2 (spray A) spots with R_F's corresponding to calotropagenin, calactinic acid, and methyl calactinate were detected.

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DEPARTMENT OF CHEMISTRY, UNIVERSITY COLLEGE,
SINGLETON PARK, SWANSEA.

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