817. *Cardenolides. Part IV.*[1] *Degradative Studies of Calactinic Acid.*

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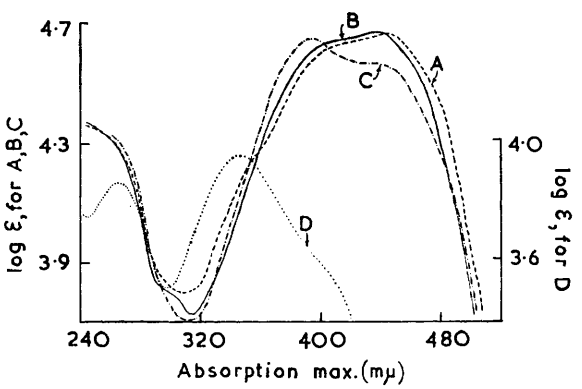
The bis-2,4-dinitrophenylhydrazone of 4-hydroxy-2-oxopentanal has been identified as the major product when 2,4-dinitrophenylhydrazine reacts with the compounds produced by acid-catalysed hydrolysis of calactinic acid. Pyrolysis of calactinic acid yields (−)-tetrahydro-2-methyl-4-oxofuran as volatile product.

THE heart poisons obtained from *Calotropis procera* R. Br. exhibit features that have not been observed in other natural cardenolides. There is evidence of thiazoline and thiazolidine fragments in uscharin [2] ($C_{31}H_{41}NO_8S$) and voruscharin [3,4] ($C_{31}H_{43}NO_8S$), respectively; the aglycone calotropagenin ($C_{23}H_{32}O_6$) which is common to all the "glycosides" [5] is novel.[6,7] The "glycosidic" fragments in the related compounds uscharidin ($C_{29}H_{38}O_9$), calotropin ($C_{29}H_{40}O_9$), calotoxin ($C_{29}H_{40}O_{10}$), and calactin ($C_{29}H_{40}O_9$) undergo transformations that have led Hesse *et al.*[8] to propose that, in uscharidin, a methylreductic acid unit replaces the carbohydrate function normally attached to the steroid 3-hydroxyl group in cardiac glycosides. Although there have been suggestions relating to structure,[8] the molecular structure of none of these constituents of *C. procera* is defined. Further evidence on the nature of the "glycosidic" fragments and their transformation products has now been sought in the hydrolysis and pyrolysis of calactinic acid.



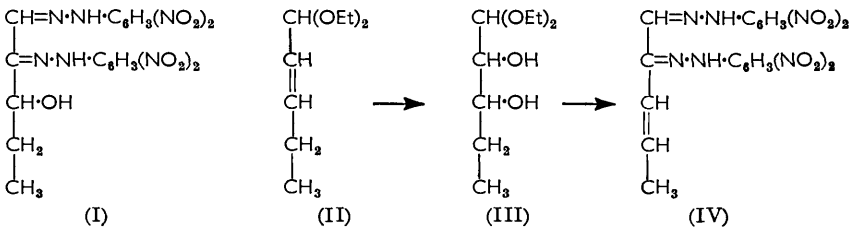
Ultraviolet absorption spectra of (A) D-glucose bis-2,4-dinitrophenylosazone and of the bis-2,4-dinitrophenylhydrazones of (B) (+)-4-hydroxy-2-oxopentanal, and (C) butane-2,3-dione. (D) D-glucose, mono-2,4-dinitrophenylhydrazone. All in ethanol.

In an earlier investigation the conversion of calactin into calactinic acid was described and it was shown [6] that calactinic acid was readily hydrolysed by dilute sulphuric acid to calotropagenin, carbon dioxide, and a C_5 fragment which was isolated as a bis-2,4-dinitrophenylhydrazone, $C_5H_8O,2(C_6H_4N_4O_4)$, $[\alpha]_D^{24}$ +250°. The ultraviolet absorption spectrum of this compound, in ethanol, showed a broad band at 400—450 mμ (log ε 4·6) and differed from the spectra of bis-2,4-dinitrophenylhydrazones that have been described.[9] Bis-2,4-dinitrophenylhydrazones with unconjugated groups have the normal characteristics of the individual chromophores.[9] We thought that the calactinic acid derivative might

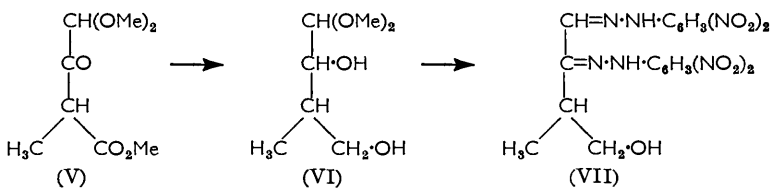
[1] Part III, *J.*, 1959, 85.
[2] Hesse, Reicheneder, and Eysenbach, *Annalen*, 1939, **537**, 67; Hesse and Gamp, *Chem. Ber.*, 1952, **85**, 933; Hesse and Mix, *Annalen*, 1959, **625**, 146.
[3] Hesse and Lettenbauer, *Angew. Chem.*, 1957, **69**, 392.
[4] Hesse and Ludwig, *Annalen*, 1960, **632**, 158.
[5] Hesse, Reicheneder, Heuser, and Hutz, *Annalen*, 1950, **566**, 130.
[6] Hassall and Reyle, *Chem. and Ind.*, 1956, 487; Hassall and Reyle, ref. 1.
[7] Geiger, Hesse, Lettenbauer, and Schildknecht, *Naturwiss.*, 1957, **44**, 328.
[8] Hesse and Lettenbauer, *Annalen*, 1959, **623**, 142, contains collected references.
[9] Jones and Hancock, *J. Amer. Chem. Soc.*, 1960, **82**, 105.

be an osazone, and this was supported by very close similarity to the spectra of the 2,4-dinitrophenylosazones of simple hexose and pentose sugars (Figure and Table 1). This postulate, together with the evidence of optical activity and the hydroxyl band at 3575 cm.$^{-1}$ in the infrared absorption spectrum, indicated the three possible structures (I), (VII), and (X).

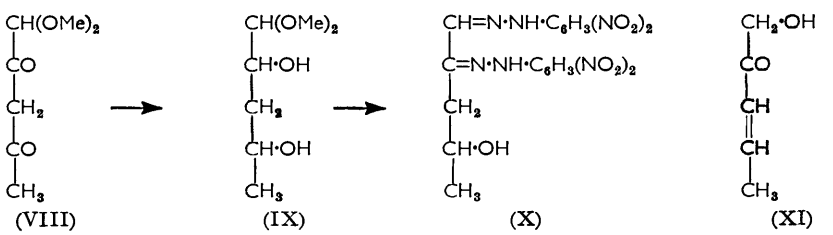
As the small amounts of material available made degradation impracticable, the identity of the 2,4-dinitrophenylosazone was established by comparison with synthetic material. Attempts to prepare the osazone (I) by hydroxylation of 1,1-diethoxypent-2-ene (II) to the diol (III), followed by mild acid-catalysed hydrolysis and treatment with 2,4-dinitrophenylhydrazine, gave the dehydration product (IV). As the derivative of the natural product, and its precursor, had been subjected to similar conditions without dehydration, the synthetic and naturally derived compounds must differ. Elimination of a hydroxyl group has been observed in a related case in which treatment of 3-hydroxy-3-methylbutan-2-one with 2,4-dinitrophenylhydrazine gave the derivative of 3-methylbut-3-en-2-one.[10]



The (±)-osazone (VII) was prepared by reduction of methyl γγ-dimethoxy-α-methyl-β-oxobutyrate (V) with lithium aluminium hydride to the diol (VI), and treatment of this with the arylhydrazine.



Synthesis of the (±)-2,4-dinitrophenylosazone (X) involved hydrogenation (Raney nickel) of 1,1-dimethoxypentane-2,4-dione (VIII) to the diol (IX), followed by hydrolysis and condensation with 2,4-dinitrophenylhydrazine.



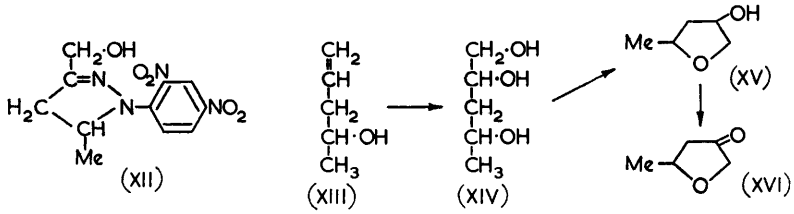
The synthetic isomer (VII) had properties that were very different from those of the osazone derived from calactinic acid. However, the infrared spectrum of compound (X) in a potassium bromide disc was so similar to that of the product from calactinic acid that it appeared likely that an optically active and a racemic form of the same compound were being compared. Both of these products were, therefore, dehydrated in the presence of

[10] Timmons, *J.*, 1957, 2613.

toluene-*p*-sulphonic acid: they gave the same unsaturated osazone (IV). This identifies the product derived from calactinic acid as the bis-2,4-dinitrophenylhydrazone (X) of (+)-4-hydroxy-2-oxopentanal.

A compound with the same properties as the bis-2,4-dinitrophenyl derivative, m. p. 259—260°, has been obtained by Hesse and his co-worker [8] by hydrolysis of a " boraxsäure," a compound which was prepared by the action of borax on uscharidin. Studies involving hydrolysis of this " boraxsäure " and cleavage of the product with periodic acid led to the suggestion that 1-hydroxypent-3-en-2-one (XI) was formed by the hydrolysis and later hydrated to 1,4-dihydroxypentan-2-one which gave rise to the osazone (X). Through the courtesy of Professor Hesse it has been possible to examine the " boraxsäure " and identify it as calactinic acid.[11] Our evidence confirms the structure (X) proposed for the bis-2,4-dinitrophenyl derivative obtained from it. However, the suggestion that the 4-hydroxyl group is introduced by hydration is now excluded by the fact that the bis-2,4-dinitrophenyl derivative derived from calactinic acid is optically active.

Pyrolysis of calactinic acid, followed by treatment of the volatile product with 2,4-dinitrophenylhydrazine, yields the single compound $C_{11}H_{12}N_4O_5$. A trace of the same product is formed during the acid-catalysed hydrolysis of calactinic acid. Hesse *et al.* also obtained this compound from " boraxsäure " and have suggested [8,12] that it is 1-(2,4-dinitrophenyl)-3-hydroxymethyl-5-methyl-2-pyrazoline (XII), formed by cyclisation of the 2,4-dinitrophenylhydrazone of the unsaturated ketol (XI). Although such a cyclisation is known [13] with αβ-unsaturated hydrazones and phenylhydrazones there is no evidence that it may occur with unsaturated 2,4-dinitrophenylhydrazones under mild conditions. Hesse *et al.* mentioned two examples [14,15] in support of their proposal but it has been possible to show [16] that no 2-pyrazoline was formed in either of these cases. There is evidence which unambiguously excludes the structure (XII) for the derivative $C_{11}H_{12}N_4O_5$. The compound is optically active and has an NH stretching frequency at 3300 cm.$^{-1}$ in the infrared spectrum.[17] The ultraviolet absorption spectrum, in ethanol, shows a band at 355 mμ (ε 22,000) and in ethanolic sodium hydroxide there is a secondary maximum at 520 mμ which is not diminished on storage for 90 minutes.[17] This spectroscopic evidence suggests that the compound is the 2,4-dinitrophenylhydrazone of an unconjugated ketone. As this ketone was probably related in structure to the precursor of the osazone (X), it seemed possible that it was one enantiomer of tetrahydro-2-methyl-4-oxofuran (XVI). This has been confirmed by synthesis.



(+)-Pent-4-en-2-ol [18] (XIII) was hydroxylated to (+)-pentane-1,2,4-triol (XIV) with potassium permanganate. The ketone (XVI) was then prepared by acid-catalysed cyclisation of the triol to the alcohol (XV), followed by oxidation. The 2,4-dinitrophenylhydrazone (m. p. 132—134°, $[\alpha]_D^{18}$ −12° ± 2°) had the same properties, including infrared absorption, as the derivative from natural sources.

[11] Crout, Curtis, and Hassall, unpublished work.
[12] Lettenbauer and Zaman, *Annalen*, 1959, **625**, 140.
[13] Elderfield's " Heterocyclic Compounds," Wiley and Sons Inc., New York, 1950, Vol. V, p. 63.
[14] Nazarov, Vartanyan, and Matsoyan, *J. Gen. Chem.* (*U.S.S.R.*), 1955, **25**, 1111.
[15] von Profft, Runge, and Jumar, *J. prakt. Chem.*, 1954, **1**, 57.
[16] Curtis, Hassall, and Weatherston, *J.*, 1962, 3831.
[17] Jones, Holmes, and Seligman, *Analyt. Chem.*, 1956, **28**, 191.
[18] Levene and Haller, *J. Biol. Chem.*, 1929, **81**, 425.

Experimental

"Deactivated" alumina refers to Spence's type H, washed in turn with an excess of dilute nitric acid, water (until neutral), and methanol, and dried at room temperature in air. Activated alumina was prepared by heating deactivated material at 120° for 12 hr. Ultraviolet absorption spectra were determined for ethanol solutions with Beckman DU and Unicam S.P. 500 spectrophotometers. Infrared spectra were measured with a Grubb–Parsons GS2 spectrometer for potassium bromide discs. Paper chromatography was carried out on Whatman No. 1 paper impregnated with formamide from a 30% acetone solution and irrigated with di-isopentyl ether saturated with formamide.[8] Vapour-phase chromatograms were obtained on a Perkin-Elmer Fraktometer 116 with 2-m. columns and hydrogen as carrier.

Hydrolysis of Calactinic Acid.—The method is similar to that described by Hassall and Reyle.[6] Calactinic acid (936 mg.) was heated under reflux in methanol (75 ml.) and 0·1N-sulphuric acid (75 ml.) for 5 hr. The solution was left overnight, the methanol was removed *in vacuo*, and the residue was extracted with (5 : 1) chloroform–ethanol (5 × 20 ml.) (to remove the genin fraction). The aqueous layer was added to 2,4-dinitrophenylhydrazine [1 g. in 100 ml. of 3N-sulphuric acid, diluted with water (200 ml.)] at *ca.* 70° and left for 7 days. Precipitated solid (740 mg.) was collected and dried.

This crude material was introduced in benzene (3 l.) on an alumina column (1 kg.; "deactivated"; 30 × 6 cm.) and eluted with benzene. Fast-running material was collected (3 l.) and gave a yellow residue (83 mg.). This was purified by chromatography over highly active alumina ("deactivated," heated over a free flame) in dichloromethane. The early fractions were combined to give a yellow residue (9·4 mg.). Paper chromatography showed that the major component was the 2,4-dinitrophenylhydrazone of tetrahydro-2-methyl-4-oxofuran (XVI) (see below), with traces of two other compounds. The main band gave the *bis-2,4-dinitrophenylhydrazone* (X) of (+)-4-hydroxy-2-oxopentanal as plates (70 mg.), m. p. 259—260° (decomp.), $[\alpha]_D^{24}$ +250° ± 5° (*c* 0·208 in acetone), $\lambda_{max.}$ 410, 438 mμ (log ε 4·63, 4·66) (Found: C, 42·6; H, 3·4; N, 23·45; O, 29·7; *C*-Me, 3·3. $C_{17}H_{16}N_8O_9$ requires C, 42·8; H, 3·4; N, 23·5; O, 30·2; 1*C*-Me, 3·2%). Elution with benzene–methanol (200 : 1) gave a yellow *substance* (62 mg.) which crystallised from chloroform and then methanol as orange-yellow prisms (16 mg.), m. p. 172—173°, $[\alpha]_D^{24}$ 0° (*c* 0·257 in acetone): $\lambda_{max.}$ 270, 366 mμ (log ε 4·46, 4·41) (Found: C, 44·9; H, 3·6; N, 24·0; *C*-Me, 3·9. $C_{17}H_{14}N_8O_8$ requires C, 44·5; H, 3·1; N, 24·4; 1*C*-Me, 3·3%). Elution with benzene–methanol (9 : 1) gave a further yellow product (185 mg.) which crystallised from ethanol as needles of *calotropagenin 2,4-dinitrophenylhydrazone*, m. p. 225°, $\lambda_{max.}$ 265 (inflexion), 361 mμ (log ε 3·92, 4·32) (Found: C, 58·0; H, 6·2; N, 9·4. $C_{29}H_{36}N_4O_{10}$ requires C, 58·0; H, 6·0; N, 9·3%).

1,1-*Diethoxypentane-2,3-diol* (III).—1,1-Diethoxypent-2-ene[19] (53·6 g.; b. p. 72—74°/30 mm.) was stirred in water (375 ml.) and treated dropwise at 3—4° with aqueous potassium permanganate (54 g., in 950 ml. of water) during 1 hr. The resulting gel was kept for 2 hr., then heated on a steam-bath to coagulate the manganese dioxide. After filtration the liquid was saturated with anhydrous potassium carbonate (600 g.) and extracted with ether (4 × 100 ml.). Distillation gave the 1,1-*diethoxypentane-2,3-diol* (III) (13·8 g.), b. p. 100—102°/2 mm., n_D^{29} 1·4328 (Found: C, 56·2; H, 10·7; O, 33·6. $C_9H_{20}O_4$ requires C, 56·2; H, 10·5; O, 33·3%).

Bis-2,4-dinitrophenylhydrazone (IV) *of 2-Oxopent-3-enal.*—The acetal (III) (1·01 g.) in ethanol (10 ml.) was added to a solution of 2,4-dinitrophenylhydrazine [(2 g. in 3N-sulphuric acid (200 ml.), diluted with water (400 ml.)] at 70° and left for 7 days. The crude *hydrazone* (IV) was collected (2·19 g.) and chromatographed in benzene on alumina as described above. Only one product (2·04 g.) was obtained. Recrystallisation from ethyl acetate–benzene gave red needles (974 mg.), m. p. 233—234°, $\lambda_{max.}$ 402, 435 (log ε 4·627, 4·625) (Found: C, 44·6; H, 3·5; N, 24·3; O, 27·9. $C_{17}H_{14}N_8O_8$ requires C, 44·5; H, 3·1; N, 24·4; O, 28·0%).

4,4-*Dimethoxy-2-methylbutane-1,3-diol* (VII).—Methyl γγ-dimethoxy-α-methyl-β-oxobutyrate[20] (V) (10·9 g.) was added with stirring to lithium aluminium hydride (3·8 g.) in ether (100 ml.). After 3 hr. damp ether was added, then a slurry of potassium hydrogen tartrate (20 g.) in water (20 ml.). The mixture was stirred and then extracted with ether for 24 hr. The ether extract yielded the *diol* (VII) (5·8 g.), b. p. 98°/0·8 mm., n_D^{28} 1·4471 (Found: C, 50·4;

[19] Kuhn and Grundmann, *Ber.*, 1937, **70**, 1894.
[20] Royals and Robinson, *J. Amer. Chem. Soc.*, 1956, **78**, 4161.

H, 9·9. $C_7H_{16}O_4$ requires C, 51·2; H, 9·8%). This was highly hygroscopic and although it distilled over a narrow range it gave bad analyses for carbon.

Bis-2,4-dinitrophenylhydrazone (VII) *of 4-Hydroxy-3-methyl-2-oxobutanal.*—The foregoing acetal (VII) (500 mg.) was hydrolysed and treated with 2,4-dinitrophenylhydrazine solution, to give the crude *hydrazone* (750 mg.). Chromatography of this product (150 mg.) gave a main fraction that crystallised from benzene as needles (64 mg.), m. p. 271—272°, $\lambda_{max.}$ 352, 403—440 mμ (log ε 4·38, 4·46—4·43) (Found: C, 42·4; H, 3·7; N, 22·9. $C_{17}H_{16}N_8O_9$ requires C, 42·8; H, 3·4; N, 23·5%).

1,1-*Dimethoxypentane-2,4-dione* (VIII).—This compound was prepared by a method analogous to that used for 1,1-dimethoxy-3-methylpentane-2,4-dione.[21] Methyl dimethoxyacetate [22] (30 g.) was added dropwise with shaking to dry sodium methoxide (from sodium, 5·2 g.). After 15 min., dry acetone (13 g.) was slowly added with shaking. After a further 90 minutes' shaking at 29°, ice–water (100 ml.) was added and the mixture was extracted with ether (2 × 40 ml.). The aqueous layer was acidified with 18% hydrochloric acid and extracted with ether (3 × 50 ml.) which was then washed and dried. Distillation gave 1,1-dimethoxypentane-2,4-dione (11·5 g.), b. p. 111—113°/30 mm., n_D^{29} 1·4542 (Royals and Robinson [20] give b. p. 73°/5 mm., n_D^{25} 1·4518). The compound gave a deep red colour with ferric chloride. Shaking it with saturated aqueous cupric acetate in dichloromethane and evaporating the organic layer gave a *copper complex* that recrystallised from light petroleum (b. p. 40—60°)–dichloromethane as green needles, m. p. 122° [Found: C, 44·1; H, 5·7; Cu, 15·5. $(C_7H_{11}O_4)_2Cu$ requires C, 44·0; H, 5·8; Cu, 16·6%].

(±)-1,1-*Dimethoxypentane-2,4-diol* (IX).—The preceding diketone (16·01 g.), was left in absolute methanol (150 ml.) overnight with freshly prepared Raney nickel W2 [23] (*ca.* 50 g.), then heated under reflux for 12 hr. (cf. Mozingo *et al.*[24]). The ferric chloride reaction was then negative. The solution was filtered, concentrated *in vacuo*, and distilled, to give the very hygroscopic (±)-*diol* (11·5 g.), b. p. 91—92°/1 mm., n_D^{29} 1·4385 (Found: C, 50·4; H, 9·7. $C_7H_{16}O_4$ requires C, 51·2; H, 9·8%).

(±)-*Bis-2,4-dinitrophenylhydrazone* (X) *of 4-Hydroxy-2-oxopentanal.*—The acetal (IX) (500 mg.) was heated on the water-bath with 1% v/v sulphuric acid (50 ml.) and ethanol (5 ml.) for 4 hr., then added to 2,4-dinitrophenylhydrazine (1 g.) in 3N-sulphuric acid (100 ml.) and water (200 ml.) at 70° and left to cool. After 2 days the red-orange solid was collected and dried (730 mg.). This was passed in benzene (5 l.) on to deactivated alumina (1 kg.) and eluted with benzene as above. After the first eluate (25 l.) the major band (12 l.) was concentrated (1·5 l.) and passed down a similar column. The eluate (30 l.) was concentrated to give the (±)-hydrazone (X) as clusters of needles (from benzene) (221 mg.), m. p. 265—267°, $\lambda_{max.}$ 410, 438 mμ (log ε 4·63, 4·65). No depression of m. p. was observed with the (+)-hydrazone from calactinic acid and the ultraviolet absorption spectra were coincident. The infrared absorption spectra showed small differences (cf. Letterbauer *et al.*[12]), as also did the crystal form.

Bis-2,4-dinitrophenylhydrazone (IV) *of 2-Oxopent-3-enal.*—(*a*) *From the* (±)-*hydrazone* (X). The preceding hydrazone (98 mg.) and toluene-*p*-sulphonic acid (10 mg.) were heated in toluene (150 ml.) under reflux for 1 hr. The solution was cooled, filtered, and passed on to deactivated alumina (40 × 2 cm.) in benzene. Elution with benzene gave a main band which gave a crude product (84 mg.). This was crystallised twice from ethyl acetate to give the *bis-2,4-dinitrophenylhydrazone* (IV) of 2-oxopent-3-enal as orange needles (16·5 mg.), m. p. 228—229°, $\lambda_{max.}$ 400, 435 mμ (log ε 4·47, 465) (Found: C, 45·2; H, 3·2; N, 24·0; O, 28·3. $C_{17}H_{14}N_8O_8$ requires C, 44·5; H, 3·1; N, 24·4; O, 27·9%).

(*b*) *From* (+)-*hydrazone* (X). The (+)-hydrazone (27·2 mg.) was treated as stated under (*a*). Chromatography (twice) on alumina and crystallisation from ethyl acetate gave the same unsaturated hydrazone (5·9 mg.), m. p. and mixed m. p. 227—229° (Found: C, 44·5; H, 3·1%); the ultraviolet and infrared absorption spectra were identical with those of the sample described under (*a*).

This hydrazone was not identical with that, m. p. 233—234°, of 2-oxopent-3-enal (IV), produced by spontaneous dehydration of 1,1-diethoxypentane-2,3-diol (III). Although no depression of m. p. was observed the infrared spectra were dissimilar. The derivative produced

[21] Helferich and Russe, *Ber.*, 1923, **56**, 763.
[22] McElvain, Mirviss, and Stevens, *J. Amer. Chem. Soc.*, 1951, **73**, 3807.
[23] *Org. Synth.*, Coll. Vol. III, 1955, p. 181.
[24] Mozingo, Spencer, and Folkers, *J. Amer. Chem. Soc.*, 1944, **66**, 1859.

by spontaneous dehydration was not affected by toluene-*p*-sulphonic acid in boiling toluene and is probably a different geometrical isomer.

Preparation of Monosaccharide 2,4-*Dinitrophenylosazones*.—The following general method was used. The monosaccharide (900 mg.) and a 5% solution (15 ml.) of 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid were heated overnight on the steam-bath. The precipitate was collected and extracted (Soxhlet) in turn with benzene and ethyl acetate. The residual solid crystallised from ethanol. Details are collected in Table 1, and some examples of ultraviolet absorption spectra are given in the Figure. These results make it clear that the product from the hydrolysis of calactinic acid could not be a bis-2,4-dinitrophenylhydrazone with isolated groupings [9] and related to a monosaccharide.

TABLE 1.

Bis-2,4-dinitrophenylosazones of sugars, etc.

Sugar	Form	M. p.[1]	$\lambda_{max.}$ (mμ) (in EtOH)	$\log_{10}\varepsilon$	Found (%) C	H	N
D(+)-Glucose	Red needles	262° [2]	405—440	4·61—4·66	—	—	—
D(+)-Galactose	Orange needles	229—235 [3]	405—440	4·58—4·60	40·3	3·6	20·8 [8]
L(+)-Arabinose	Red prisms [4]	259—260	415—440	4·58—4·60	40·4	3·5	21·6 [9]
D(+)-Xylose	Red needles	229	405—440	4·60—4·62	40·2	3·4	22·8 [9]
L(−)-Rhamnose	Rectangular prisms	244	410—440	4·63—4·63	41·2	3·2 [10]	—
Glyoxal	Orange needles [5]	320	392—440 [6]	4·62—4·64	—	—	—
Butane-2,3-dione	Prisms [5]	319	395—430 [7]	4·64—4·57	—	—	—

[1] With decomp. [2] Ref. 25, m. p. 263—267°. [3] Ref. 26, m. p. 185° (clearly impure). [4] From EtOAc. [5] From $CHCl_3$. [6] Ref. 9, $\lambda_{max.}$ 391, 438 mμ ($\log\varepsilon$ 4·17, 4·43). [7] Ref. 9, $\lambda_{max.}$ 393, 435 mμ ($\log\varepsilon$ 4·69, 3·97). [8] $C_{18}H_{18}N_8O_{12}$ requires C, 40·2; H, 3·4; N, 20·8%. [9] $C_{17}H_{16}N_8O_{11}$ requires C, 40·2; H, 3·2; N, 22·05%. [10] $C_{17}H_{16}N_8O_{10}$ requires C, 41·5; H, 3·3%.

TABLE 2.

2,4-Dinitrophenylhydrazones of sugars.

Sugar	Form	M. p.*	$\lambda_{max.}$ (mμ)	$\log_{10}\varepsilon$	Found (%) C	H	Formula	Reqd. (%) C	H
D(+)-Glucose	Yellow needles	118° †	266, 345	3·87, 3·95	—	—	—	—	—
D(+)-Xylose	Yellow needles	162° ‡	266, 350	3·77, 3·87	—	—	—	—	—
L(+)-Arabinose	Brown needles	142—144	269, 352	3·96, 4·01	40·0	4·1	$C_{11}H_{14}N_4O_8$ §	40·0	4·3
L(−)-Rhamnose	Yellow plates	167	262, 347	3·88, 4·09	41·7	4·6	$C_{11}H_{14}N_4O_7$	42·0	4·5

* With decomp. † Ref. 27, m. p. 122—124°. ‡ Ref. 27, m. p. 162. § Found: N, 16·6. Reqd.: N, 17·0%.

Preparation of Monosaccharide 2,4-*Dinitrophenylhydrazones*.—The method of Lloyd and Doherty [27] was used to prepare some of these compounds for examination of their ultraviolet absorption spectra. The results are collected in Table 2.

Pyrolysis of Calactinic Acid.—Calactinic acid (94 mg.) was heated in a U-tube immersed in an oil-bath under a very slow stream of nitrogen (1 bubble/sec.) at 250° (bath-temp.) for 45 min. The products were passed into a trap at −80°, then washed into a solution of 2,4-dinitrophenylhydrazine (1 g.) in 3N-sulphuric acid (250 ml.) and warmed for 20 min.; the solution was left overnight; the yellow-orange precipitate was collected and dried (17·2 mg.). Paper chromatography showed two spots, of R_F 0·54 and 0·00. Chromatography in dichloromethane over alumina (activated at 180°) (10 × 1 cm.) gave a main yellow band and a slower orange band.

Material from the yellow band was crystallised twice from methanol, to give the 2,4-*dinitrophenylhydrazone* (cf. XVI) of (−)-tetrahydro-2-methyl-4-oxofuran (12·9 mg.), m. p. 135—136° (corr.), $[\alpha]_D^{25}$ −13° ± 2° (*c* 0·111 in dichloromethane), $\lambda_{max.}$ 355 mμ ($\log\varepsilon$ 4·35) (Found: C, 46·7; H, 4·5; N, 20·5; O, 28·3. $C_{11}H_{12}N_4O_5$ requires C, 47·1; H, 4·3; N, 20·0; O, 28·6%), $\nu_{max.}$ 3300 $cm.^{-1}$ (NH), R_F on paper, 0·72.

Material from the orange band crystallised from dichloromethane, giving the (+)-4-hydroxy-2-oxopentanal 2,4-dinitrophenylhydrazone as red needles (1·2 mg.), $[\alpha]_D^{20}$ +170° ± 5°

[25] Wolfrom and Arsenault, *J. Org. Chem.*, 1960, **25**, 205.
[26] Neuberg and Strauss, *Arch. Biochem.*, 1946, **11**, 457.
[27] Lloyd and Doherty, *J. Amer. Chem. Soc.*, 1952, **74**, 4214.

(*c* 0·048 in EtOH), m. p. 256—259° alone or mixed with the sample obtained after hydrolysis of calactinic acid and with superimposable ultraviolet- and infrared-absorption curves.

(+)*Pentane*-1,2,4-*triol* (XIV).—Pent-4-en-2-ol was prepared according to directions of Yanko *et al.*[28] and the phthalate was resolved [18] with (−)-brucine, to give an alcohol (XIII), b. p. 112—116°/760 mm., n_D^{16} 1·4270, $[\alpha]_D^{16}$ +10° ± 2° (*c* 19·5 in acetone). Levene and Haller [18] give b. p. 116—118°, $[\alpha]_D^{20}$ −6·1° in ether, for the (−)-isomer.

To (+)-pent-4-en-2-ol (6·55 g.) in water (30 ml.) at −5°, potassium permanganate (7·4 g.) in water (150 ml.) was added during 2 hr. with stirring. After 1 hour's warming on the water-bath manganese dioxide was collected and the pH of the filtrate adjusted to 7·2. Water was removed at 40°, to give a mixture of brown viscous syrup and crystalline manganese salts. Inorganic material was removed by filtration and washed with a small amount of methanol. The combined filtrates were distilled, to give (+)-*pentane*-1,2,4-*triol* (XIV) (1·11 g.), b. p. 125—126°/0·4 mm., α_D^{16} +15° ± 2° (*c* 24·4 in acetone). Wagner [29] gives b. p. 180°/27 mm. for the (±)-form.

Before optically active materials were used in subsequent reactions the optically inactive compounds were subjected to the same reactions. In the following paragraphs experiments on the optically inactive material are described in detail.

Tetrahydro-4-*hydroxy*-2-*methylfuran* (XV).—Pentane-1,2,4-triol (XIV) (8·0 g.) and toluene-*p*-sulphonic acid (79 mg.) were distilled during 2·5 hr. at 60°/1 mm. Redistillation gave crude tetrahydro-4-hydroxy-2-methylfuran (3·9 g.), b. p. 98—100°/30 mm. (Reppe [30] gives b. p. 90—91°/20 mm., but no analysis) (Found: C, 59·1; H, 9·9. Calc. for $C_5H_{10}O_2$: C, 58·8; H, 9·9%). Vapour-phase chromatograms on polyethylene glycol at 128° showed that this compound was not completely homogeneous but contained an unsaturated impurity [infrared absorption at 1653 cm.$^{-1}$ (CH=CH)] which could not be removed by fractional distillation.

(+)-Pentane-1,2,4-triol (1 g.) was dehydrated in 1 hr., as above, to give (−)-tetrahydro-4-hydroxy-2-methylfuran (192 mg.), b. p. 90—92°/25 mm., $[\alpha]_D^{20}$ −13° ± 2° (*c* 21·8 in ether). This material also contained an unsaturated impurity, shown by vapour-phase chromatograms as above.

Tetrahydro-2-*methyl*-4-*oxofuran* (XVI).—Crude tetrahydro-4-hydroxy-3-methylfuran (XV) (41·3 g.) in ether (100 ml.) at −10° was treated, with stirring, with sodium dichromate (68·2 g.) in concentrated sulphuric acid (45 ml.) and water (278 ml.) during 4 hr. The mixture was allowed to reach room temperature in 3 hr., then continuously extracted with ether, and the ethereal layer was worked up, to give *tetrahydro*-2-*methyl*-4-*oxofuran* (XVI), b. p. 146°/760 mm. (6·6 g.), n_D^{17} 1·4321, $\nu_{max.}$ 1751 cm.$^{-1}$ (C=O). The compound appeared to be unstable and darkened considerably within 48 hr. (Found: C, 58·9; H, 8·3. $C_5H_8O_2$ requires C, 59·9; H, 8·1%). The corresponding infrared maximum of tetrahydro-3-oxofuran [31] was at 1754 cm.$^{-1}$. The ketone (XVI) was homogeneous in vapour-phase chromatograms on didecyl phthalate at 126°. It gave a 2,4-*dinitrophenylhydrazone*, yellow needles (from methanol), m. p. 132—134°, $\lambda_{max.}$ 354 mμ (log ε 4·35) (Found: C, 47·5; H, 4·6; N, 19·5; O, 28·7. $C_{11}H_{12}N_4O_5$ requires C, 47·1; H, 4·3; N, 20·0; O, 28·6%), $\nu_{max.}$ 3310 cm.$^{-1}$ (NH). The 2,4-dinitrophenylhydrazone of tetrahydro-3-oxofuran kindly supplied by Professor Wynberg had $\lambda_{max.}$ 354 mμ (log ε 4·35), $\nu_{max.}$ at 3300 cm.$^{-1}$ (NH).

A similar oxidation gave the (−)-ketone, b. p. 142—148°/760 mm., $[\alpha]_D^{20}$ −37° ± 2° (*c* 25·2 in ether). Its 2,4-*dinitrophenylhydrazone*, yellow needles (from methanol) (10 mg. from 192 mg. of alcohol), m. p. 132—134°, $[\alpha]_D^{18}$ −12° ± 2° (*c* 1 in dichloromethane), behaved identically on paper chromatograms, and gave an identical infrared absorption spectrum and no depression of the m. p. when compared with material obtained after pyrolysis of calactinic acid.

We are grateful to the Rockefeller Foundation for a Fellowship tenable at the University College of the West Indies (R. F. C.) and British Petroleum Ltd. for a Studentship (J. W.).

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Singleton Park, Swansea. [*Received, March 2nd,* 1962.]

[28] Yanko, Mosher, and Whitmore, *J. Amer. Chem. Soc.*, 1945, **67**, 666.
[29] Wagner, *Ber.*, 1888, **21**, 3351.
[30] Reppe, *Annalen*, 1955, **596**, 113.
[31] Wynberg, *J. Amer. Chem. Soc.*, 1958, **80**, 365.