405. *Cardenolides. Part VI.*[1] *Uscharidin, Calotropin, and Calotoxin.*

By D. H. G. Crout, C. H. Hassall, and T. L. Jones.

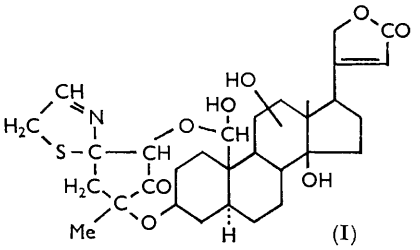
New evidence, relating in particular to the products of thermal degradation and of the action of mild alkali, leads to the structures (XIII), (XIX), and (XXVI) for the steroid " glycosides " uscharidin, calotropin, and calotoxin, derived from the plant species *Calotropis procera*.*

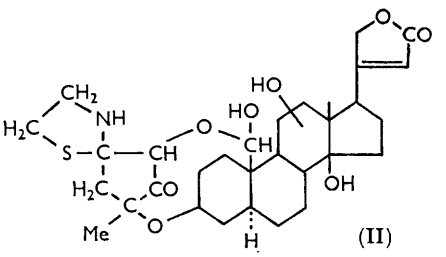
Investigations by Hesse and his co-workers [3,4] have led to the isolation of the heart poisons uscharin ($C_{31}H_{41}NO_8S$), voruscharin ($C_{31}H_{43}NO_8S$), uscharidin ($C_{29}H_{38}O_9$), calotropin ($C_{29}H_{40}O_9$), calotoxin ($C_{29}H_{40}O_{10}$), and calactin ($C_{29}H_{40}O_9$) from the plant species *Calotropis procera* R. Br. (family Asclepiadaceae). Calotropagenin, which has been assigned, tentatively, the structure (VI) [1,5] has been shown to be the aglycone common to all the glycosides.[6] Degradative evidence and partial syntheses, starting from uscharidin, have indicated that voruscharin and uscharin have thiazolidine and thiazoline fragments associated with the " carbohydrate " function in uscharidin.[4,7]

* A preliminary account of part of this work has been published.[2]

[1] Part V, Crout, Curtis, and Hassall, *J.*, 1963, 1866.
[2] Crout, Curtis, Hassall, and Jones, *Tetrahedron Letters*, 1963, 63.
[3] Hesse, Reicheneder, and Eysenbach, *Annalen*, 1939, **537**, 67.
[4] Hesse and Ludwig, *Annalen*, 1960, **632**, 158.
[5] Hassall and Reyle, *J.*, 1959, 85.
[6] Hesse, Heuser, Hütz, and Reicheneder, *Annalen*, 1950, **566**, 130.
[7] Hesse and Mix, *Annalen*, 1959, **625**, 146.

The elucidation of the structures of the carbohydrate fragments in these compounds has been complicated by their resistance to acid-catalysed hydrolysis. As a result, use has been made of thermal decomposition and alkaline hydrolysis to cleave the glycosides. Although these reactions have taken place under relatively mild conditions, they evidently allow significant modification of the carbohydrate fragment. The cleavage reactions have been interpreted by Hesse and his co-workers in terms of structures (I)—(V), respectively,


(I)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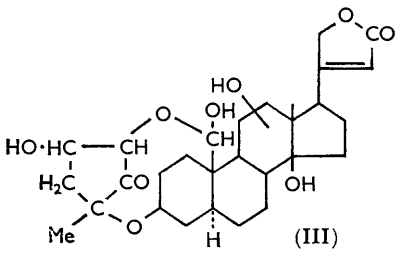
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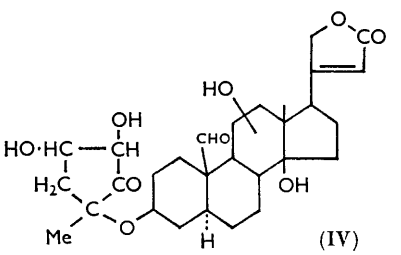
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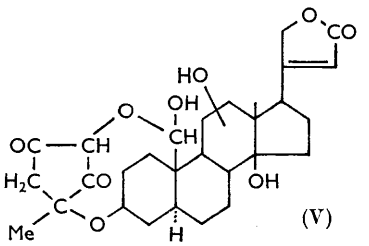
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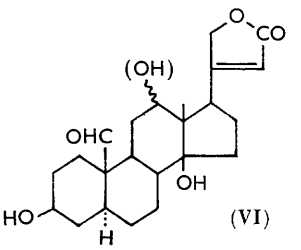
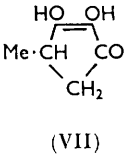

(VI)

for uscharin,[7] voruscharin,[4] calotropin,[8] calactin,[9] and uscharidin.[8] New evidence relating to the action of mild alkali on uscharidin, has thrown doubt on the formulation of this compound and has led us to reconsider the structures of the other glycosides in this series.

Uscharidin and Calactin.—Uscharidin has been obtained both from the mixture of glycosides in *Calotropis procera* and by treatment of uscharin and voruscharin with mercuric chloride. The molecular formula, $C_{29}H_{38}O_9$, indicates that calotropagenin (VI) is combined with a fragment $C_6H_7O_3$, presumably attached to the oxygen function at position 3 of the steroid nucleus. Hesse and Lettenbauer [8] have suggested that this fragment contains two carbonyl groups, and we concur with this suggestion. It is based on spectroscopic evidence, on the formation of derivatives, and on the properties of the reduction products of uscharidin. Their suggestion that these groups are incorporated in a methylreductic acid unit (VII) was influenced by the observation that calotropin (= dihydro-uscharidin) gave rise to methylreductic acid through pyrolysis and treatment with alkali.[10] It was considered [8] that the 1,3-dioxo-group in uscharidin could undergo cleavage with borax solution or with alumina to give calactinic acid (VIII). Other reactions, such as the behaviour of uscharidin and its reduction products with periodic

[8] Hesse and Lettenbauer, *Annalen*, 1959, **623**, 142.
[9] Hesse, Fasold, and Geiger, *Annalen*, 1959, **625**, 157.
[10] Hesse, Hertel, and Mix, *Annalen*, 1959, **625**, 174.

acid,[11] were interpreted in terms of structure (V) but did not provide unequivocal support. Recently, it has been shown [1] that the structure of calactinic acid is not (VIII) but (IX). Evidently this requires a revision of the structure of uscharidin; formulation (V) cannot account for the formation of acid (IX) through the action of very mild alkali.



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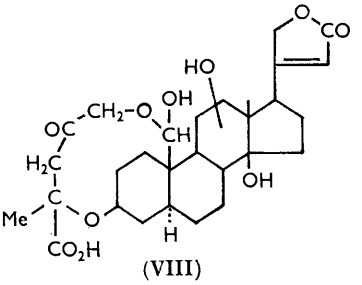
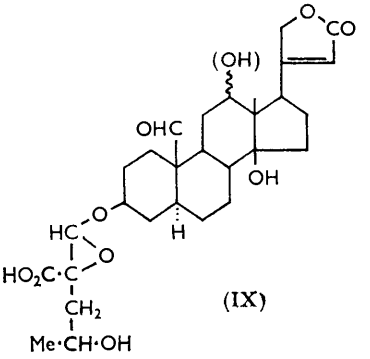
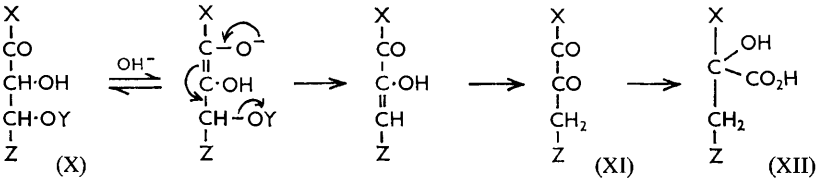


(VIII)



(IX)

There appeared to be some similarity between the conversion of uscharidin into calactinic acid and the formation of an isosaccharinic acid, by the alkaline degradation of a hexose. This led us to investigate the possibility that the reaction leading to calactinic acid was subject to the specific catalysis by calcium ions which is characteristic of



(X) (XI) (XII)

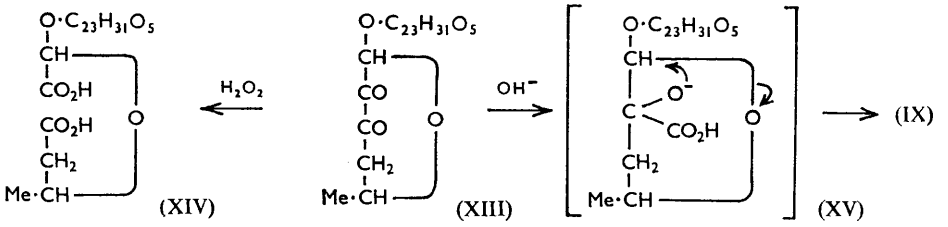
isosaccharinic acid formation.[12] O'Meara and Richards [13] have shown that this catalysis is associated with the benzilic acid-type rearrangement of the intermediate α-dicarbonyl compound (XI) which arise through elimination of the hydroxyl group β to the carbonyl group in a hexose (X).

We have found that conversion of uscharidin into calactinic acid is accelerated by calcium ions. Moreover, the catalytic effect was comparable with that involved in the rearrangement of glyoxal to glycollic acid under similar conditions.[13] With 0·005 molar solution the ratio of half reaction time of the catalysed process : half reaction time of the uncatalysed process, was 4·1 and 3·7 for the calactinic acid and the glycollic acid reactions, respectively.

Oxidation with hydrogen peroxide at pH 8·7 and room temperature provided further evidence of a 1,2-diketone unit.[14] There was an uptake of 1 mol. of reagent with the formation of a dibasic acid which, only after acid-catalysed hydrolysis, released β-hydroxybutyric and crotonic acid. This leads to the formulation of uscharidin as (XIII). The conversion of uscharidin into calactinic acid (IX) is interpreted as proceeding through the intermediate (XV). Although there is no direct analogy for the formation of a glycidic acid derivative in this way, similar reactions leading to epoxides and, more particularly, sugar epoxides [15,16] are well established.

[11] Hesse and Geiger, *Annalen*, 1959, **625**, 161.
[12] Machel and Richards, *J.*, 1960, 1924, 1932.
[13] O'Meara and Richards, *J.*, 1960, 1944.
[14] Hassall, *Org. Reactions*, 1957, **9**, 73.
[15] Newth, *Quart. Rev.*, 1959, **13**, 30.
[16] Peat, *Adv. Carbohydrate Chem.*, 1946, **2**, 38.

Uscharidin in aqueous methanol reacts with 1 mol. of periodic acid during 45 hours with, presumably, initial formation of the acid (XIV). However, in aqueous dioxan during five days further oxidation has been observed. Formic acid, calotropagenin, and what is probably calotropagenin-19-carboxylic acid have been identified in this mixture.[11]



These products can be attributed to partial hydrolysis of the initial product of oxidation followed by slow oxidation of the glyoxylic acid [17] and the aglycone.

The relationship of calactin and uscharidin is somewhat obscure. Hesse and his co-workers [2] describe the isolation of calactin from the latex of *Calotropis procera* and the production of similar material in undefined yield by catalytic hydrogenation of uscharidin.[5] We have not been able to differentiate calactin and uscharidin (Table 1). The differences

TABLE 1.

Comparison of the properties of uscharidin and calactin.

	Uscharidin		Calactin	
	Ref. 5	Ref. 4 or present work	Ref. 5	Ref. 18
M. p. (decomp.) of solvent-free compound	290°	290°	275°	270—272°
M. p. (decomp.) of chloroform complex	195	195	190	234—246
M. p. (decomp.) of ethanol complex	204—206	208—210	220	—
M. p. (decomp.) of oxime	257	256	270	—
$[\alpha]_D$ (of solvent-free compound in MeOH or EtOH) ...	+36	+38	+48	+70

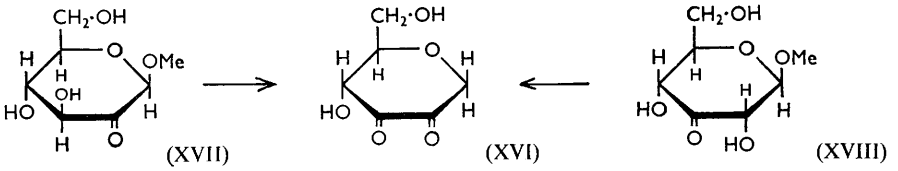
in melting point (with decomposition) and optical rotation may be attributed to differences in purity. Preparations of uscharidin, which have been shown by reversed-phase paper chromatography with chloroform–dimethylformamide to be contaminated with small quantities of calotropin and calotoxin, may have a crystalline form similar to that attributed to calactin. We have found through paper chromatography that the preparation described as calactin in our earlier publication was uscharidin contaminated with small quantities of calotropin, calotoxin, and with traces of two, as yet, undefined compounds. Paper chromatography of a sample of calactin kindly provided by Professor G. Hesse showed that this was a similar mixture in which the main component had the same R_F as uscharidin; moreover, like uscharidin, this sample was converted in good yield into calactinic acid by contact with alumina. We are in no doubt that we have had pure uscharidin in our hands but we have not been able to obtain a separate compound, calactin, from the mixture of glycosides in the root bark of *Calotropis procera*. Recently Reichstein and his co-workers [18] have described the isolation of calactin, calotropin, calotoxin, calotropagenin, and *al*-dihydrocalotropagenin, but not uscharidin, from the stems of *Pergularia extensa* (family Asclepiadaceae). These investigators have observed some constants for calactin, which are significantly different from those recorded earlier.

Calotropin.—Treatment of uscharidin with 1 mol. of sodium borohydride gave a 51% yield of calotropin.[9] As this glycoside is, like, uscharidin, derived from calotropagenin, the reduction process must involve one of the carbonyl groups in the glycosidic fragment of uscharidin. Structure (XIX) for calotropin accounts for the reaction with 0·9 mol. of

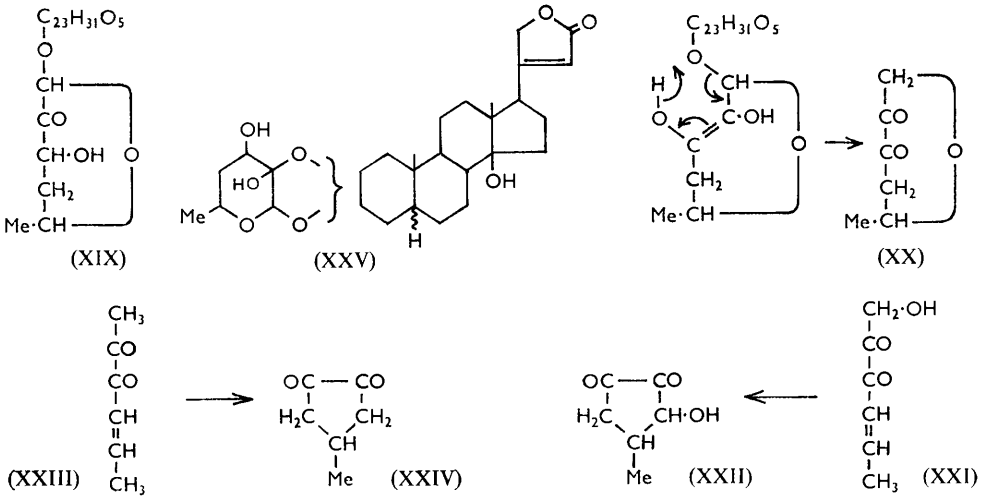
[17] Fleury and Bon-Bernatets, *J. Pharm. Chim.*, 1936, **23**, 85.
[18] Mittall, Tamm, and Reichstein, *Helv. Chim. Acta*, 1962, **45**, 907.

periodic acid during 24 hours, leading to crotonaldehyde. The formation of some calotropagenin and formic acid in this process is evidently due to side reactions, as in the case of uscharidin.

When calotropin was heated at 250° under reduced pressure, two volatile compounds were formed. The major product, $C_6H_8O_3$, was optically active, had no acidic or reducing properties, formed a bis-2,4-dinitrophenylhydrazone, and was like calotropin itself converted by alkali into the second product, which was identified as (±)-methylreductic acid (XXII) through comparison with synthetic material.[10]



We attribute structure (XX) to the major product of thermal degradation of calotropin, and the formation of this compound to a process similar to the conversion of methyl β-D-2(or 3)-gluculopyranosides (XVII, XVIII) into the diketone (XVI), a reaction which has been brought about by the action of mild alkali and by heating in neutral or even in weakly acidic media.[19] The formation of (±)-4-methylreductic acid by the action of alkali



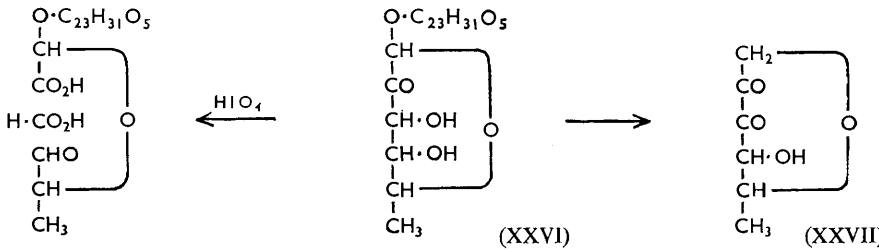
on (+)-tetrahydro-2-methyl-4,5-dioxopyran (XX) is attributed to a reaction sequence in which the intermediate (XXI) undergoes cyclisation through intramolecular Michael condensation. There is an analogy for such a reaction in the base-catalysed cyclisation of 1,5-diphenylpent-4-ene-2,3-dione to 3,4-diphenylcyclopentane-1,2-dione.[20] As it seemed possible that the phenyl substituents played an important role in this case we have studied the action of base on hex-4-ene-2,3-dione (XXIII). This compound, which was prepared by hydration of hex-4-en-1-yn-3-one with sulphuric acid and mercuric sulphate, was converted into 4-methylcyclopentan-1,2-dione (XXIV) in the presence of piperidine or dilute sodium hydroxide.

The unusual 4,6-dideoxyhexosone structure which is assigned to the carbohydrate portion of calotropin resembles that recently attributed to the cardiac glycoside

[19] Theander, *Acta Chem. Scand.*, 1958, **12**, 1887, 1897.
[20] Cragg, Dean, and Winfield, *J.*, 1959, 2431.

gomphoside (XXV), in which, however, the 2-carbonyl function has combined with an hydroxyl group of the aglycone to form a hemiketal link.[21]

Calotoxin.—The formation of uscharidin (XIII; $C_{29}H_{38}O_9$) when calotoxin ($C_{29}H_{40}O_{10}$) was heated with aluminium hydroxide [5] established the close relationship of these glycosides. This was further clarified by oxidation experiments. Although calotoxin did not react with hydrogen peroxide under the conditions that led to cleavage of the α-dicarbonyl group in uscharidin, it was oxidised by 1·6 mol. of periodic acid during 21 hours. Formic acid was produced, and the formation of lactaldehyde through periodic acid cleavage followed by acid-catalysed hydrolysis, was inferred from the observation that the bis-2,4-dinitrophenylhydrazone of methylglyoxal could be prepared from the reaction mixture. The intensity of absorption at $\lambda_{max.}$ 309 (log ε, 1·76) indicates the presence of a carbonyl group in the glycosidic function, in addition to that at position 19 of the steroid nucleus. This evidence leads to structure (XXVI) for calotoxin.



(XXVI) (XXVII)

Calotoxin undergoes thermal degradation to give an optically active compound, $C_6H_8O_4$, which behaves as a monobasic acid, gives a bis-2,4-dinitrophenylhydrazone and reduces triphenyltetrazolium chloride on warming. It has a u.v. absorption spectrum [$\lambda_{max.}$ 290 mμ (log ε 3·74)] very similar to that of the diketone (XVI).[19] The structure (XXVII) accounts for these properties and for the formation of the compound from calotoxin (XXVI) by a process similar to the thermal degradation of calotropin.

Experimental

U.v. absorption spectra were determined for ethanol solutions with a Unicam S.P. 500 spectrophotometer and an Optica CF4 recording spectrophotometer. I.r. absorption spectra were measured for potassium bromide discs with a Perkin-Elmer Infracord instrument. Vapour-phase chromatograms were obtained on a Perkin-Elmer Fraktometer 116 with 2m. columns and hydrogen as carrier.

Paper chromatography was on Whatman No. 1 paper, the following systems being used: (1), paper impregnated with formamide, irrigated with chloroform saturated with formamide; [8] (2), butan-1-ol saturated with 1·5N-ammonia.[22] 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), Bromocresol Purple (0·04% w/v) in ethanol–formalin (5 : 1) with pH adjusted to 5·0 with sodium hydroxide.[22]

Uscharidin (XIII).—Paper chromatography, using the reversed phase system No. 1 and spray reagent A, showed that the calactin prepared in the earlier study [4] contained uscharidin as the main product, contaminated with calotoxin (R_F, 0·07), calotropin (R_F, 0·52), and traces of two compounds (R_F 0·64 and 0·16) with positive Kedde reactions. (The R_F values are in relation to uscharidin taken as 1·0). Attempts to obtain uscharidin from this preparation by recrystallisation or by chromatography on alumina and on silica gel, did not yield a pure product.

Pure uscharidin was prepared through acid-catalysed hydrolysis of uscharin. The brown, friable powder (26·3 g.) obtained just before the desulphurisation step in the preparation of calactinic acid [1] was dissolved in hot ethanol (100 ml.) and cooled. The crystalline product (4·38 g.) that separated was chromatographed on alumina (160 g.), ethyl acetate and ethyl

[21] Coombe and Watson, *Proc. Chem. Soc.*, 1962, 214.
[22] Lederer and Reid, *Biochem. J.*, 1951, **50**, 60.

acetate–ethanol being used for elution. The initial eluates [ethyl acetate and ethyl acetate–ethanol (10 : 1)] contained crude uscharin (420 mg.), which crystallised from ethyl acetate as needles, $[\alpha]_D^{22}$ +26 ± 2° (*c* 0·9 in acetone), m. p. and mixed m. p. with authentic uscharin kindly supplied by Professor G. Hesse, 267—268°. Uscharin (2·2 g.) in methanol (50 ml.) and 2N-sulphuric acid (50 ml.) was refluxed under nitrogen for 4 hr. The chloroform extract of the mixture was concentrated to 10 ml. and cooled to give uscharidin–chloroform complex (1·4 g.) as prisms, m. p. and mixed m. p. 194—196°; the solvent-free uscharidin had m. p. 290°, $[\alpha]_D^{20}$ +38 ± 2° (*c* 0·9 in MeOH) (Found: C, 65·2; H, 7·1. Calc. for $C_{29}H_{38}O_9$: C, 65·6; H, 7·2%). Uscharidin in methanol–water reacted with 1·0 mol. of 0·18M-periodic acid in 45 hr. The conditions used in this oxidation have been defined in the previous Paper.[1]

Reactions of Uscharidin.—(a) *With sodium hydroxide–calcium hydroxide.* Aqueous sodium hydroxide was added to a solution of uscharidin (0·0054M, 5 ml.) in dioxan–water (1 : 4) to bring the final volume to 10 ml. and to 0·005N with respect to alkali. The water used for the preparation of solutions had been freed from dissolved oxygen. The mixture was kept at 22° and portions (1·0 ml.) were withdrawn at intervals, added to a known excess of 0·01N-hydrochloric acid and titrated under nitrogen, potentiometrically, against 0·01N-sodium hydroxide. The experiment was repeated with the addition of calcium chloride to bring the solution to 0·005M but maintaining the hydroxyl-ion concentration at 0·005N. The results of a typical experiment are shown in Table 2.

TABLE 2.

Rate of acid formation by action of alkali on uscharidin at 22°.

(A) In 0·005N-sodium hydroxide

Time (min.)	7·5	15·5	31	60	210	300
Acid (equiv./mole)	0·15	0·19	0·22	0·29	0·44	0·49

Half-reaction time ($At_{0.5}$), 320 min.

(B) In 0·005N-sodium hydroxide + 0·005M-calcium chloride.

Time (min.)	1·5	6	30	105	175	302
Acid (equiv./mole)	0·13	0·20	0·34	0·57	0·69	0·86

Half-reaction time ($Bt_{0.5}$), 78 min.

$At_{0.5}/Bt_{0.5} = 4·1$.

(b) *With hydrogen peroxide–sodium hydroxide.* Uscharidin (10·2 mg.) in methanol (5 ml.) was brought to pH 8·7, under nitrogen by addition of 0·25N-sodium hydroxide to the cell of a Radiometer titrator TTT 1a. Hydrogen peroxide (30%; 0·2 ml.) was added and the pH-stat was set to maintain a constant pH 8·7. After 4 hr. there was an uptake of 1·76 mole of sodium hydroxide per mole of uscharidin. A similar reaction with benzil consumed 1·95 mole of sodium hydroxide. It was shown by studying the change in intensity of absorption at 218 mμ that the butenolide ring in uscharidin did not react with alkali at pH 8·7.

The hydrogen peroxide consumed in this reaction was determined in a separate experiment. Uscharidin (187 mg.) in methanol (10 ml.) was treated with hydrogen peroxide (30%; 0·20 ml.) at pH 8·7. After 4 hr. in the dark under nitrogen, the solution was cooled to 0°, platinum black (5 mg.) was added, and the oxygen evolved was measured (14·3 ml.). A series of experiments without uscharidin gave 17·8 ± 0·4 ml. of oxygen. The hydrogen peroxide consumed corresponded to 0·84 mole per mole of uscharidin. In a similar experiment one mole of benzil consumed 0·91 mole of hydrogen peroxide.

Hex-4-ene-2,3-dione (XXIII).—Hex-4-en-1-yn-3-one (2,4-dinitrophenylhydrazone, m. p. 162—163°) was prepared according to Bowden *et al.*[23] In this preparation it was necessary to repeat the oxidation of hex-4-en-1-yn-3-ol several times with chromic acid to ensure that no starting material contaminated the product. This was confirmed by g.l.c., polyethylene glycol being used as stationary phase. Hex-4-ene-2,3-dione was obtained by heating the hex-4-en-1-yn-3-one (50 g.), mercuric sulphate (25 g.), and sulphuric acid (500 ml.; 10% w/v) at 100° for 1 hr. followed by steam distillation under nitrogen. The distillate was saturated with sodium chloride and extracted with ether. The residue, after removal of ether, distilled as a yellow oil, b. p. 55—56°/16 mm. G.l.c. showed the presence of one major product (relative retention time 1·25 min.) contaminated with approximately 10% of starting material (retention time, 4·3 min.) which was not removed by distillation. The major product was characterised as hex-4-ene-2,3-dione through the *bis*-2,4-*dinitrophenylhydrazone* (85% yield), orange-red

[23] Bowden, Heilbron, Jones, and Weedon, *J.*, 1946, 39.

plates (from ethanol), m. p. 248—249°; $\lambda_{max.}$ 342 and 397 mμ (log ε 4·30 and 4·31) [Found: C, 46·3; H, 3·7; O, 27·4. $C_{18}H_{16}N_8O_8$ requires C, 45·8; H, 3·4; O, 27·1%].

4-Methylcyclopentane-1,2-dione (XXIV).—Hex-4-ene-2,3-dione (50 mg.) dissolved in 2N-sodium hydroxide (1·5 ml.) and ethanol (3 ml.) to give an intensely yellow solution. Measurement of the change with time of the intensity at the maximum u.v. absorption, both for the alkaline solution ($\lambda_{max.}$ 288 mμ) and after being made acid to Congo Red with 2N-hydrochloric acid ($\lambda_{max.}$ 248—256 mμ), indicated that the optimum reaction time was about 195 min.

For the isolation of the products, hex-4-ene-2,3-dione (200 mg.) was kept at 20° in oxygen-free 2N-sodium hydroxide (6 ml.) and ethanol (12 ml.) for 195 min., and the mixture was then acidified and extracted with ether. As the gum (194 mg.) did not yield crystalline material it was allowed to react with 2,4-dinitrophenylhydrazine. The mixture of 2,4-dinitrophenylhydrazones (95 mg.) was separated into two major fractions by chromatography on alumina dichloromethane–chloroform mixtures being used for elution. The first fraction crystallised from ethanol as orange needles, m. p. and mixed m. p. with the 2,4-dinitrophenylhydrazone of synthetic 4-methylcyclopentane-1,2-dione, 234° (decomp.) [Found: C, 45·7; H, 3·8; N, 23·8. Calc. for $C_{18}H_{16}O_8N_8$: C, 45·8; H, 3·4; N, 23·7%]; the two samples had identical i.r. spectra. The second fraction, m. p. 248°, was the bis-2,4-dinitrophenylhydrazone of hex-4-ene-2,3-dione.

When hex-4-ene-2,3-dione (200 mg.) in ethanol (10 ml.) was left with piperidine (0·02 ml.) under nitrogen at 20° the optimum reaction time was 165 min. The mixture was acidified and extracted with ether. Removal of the ether left a gum (172 mg.) which was distilled to give two major fractions. The first fraction (112 mg.; b. p. 53—58°/16 mm.) was identified as starting material. The second (b. p. 90—100°/16 mm.) solidified on the condenser. The product from three similar reactions was recrystallised from benzene–light petroleum, forming plates (26 mg.); $\lambda_{max.}$ 257 mμ (log ε 3·75), m. p. 56—59° undepressed on admixture with authentic 4-methylcyclopentane-1,2-dione,[24] 59° [Found: C, 64·2; H, 7·3. Calc. for $C_6H_8O_2$: C, 64·3; H, 7·2%]. The compounds had identical i.r. absorption spectra.

Calotoxin (XXVI).—This glycoside, $\lambda_{max.}$ 309 and 217 mμ (log ε 1·76 and 4·17) [Found: C, 63·2; H, 7·7. Calc. for $C_{29}H_{40}O_{10}$: C, 63·5; H, 7·4%], was prepared by chromatography on silica gel of the residue obtained from the chloroform and methanol eluates in the last stage of the preparation of calactinic acid.[1]

When calotoxin (10·4 mg.) in methanol (5 ml.) was treated with hydrogen peroxide (0·2 ml.), as in the case of uscharidin (*vide supra*), no acid was produced. Paper chromatography (system No. 1, spray A) showed that the only compound reacting with Kedde reagent was starting material.

Periodate Oxidation of Calotoxin.—Calotoxin (2·13 g.) in chloroform–methanol–water (5 : 6 : 2; 400 ml.) was treated with periodic acid (1·12 g.) in water (100 ml.) during 24 hr. in the dark. A portion (100 ml.) was removed and steam-distilled. A small sample of the distillate was shown by paper chromatography (system No. 2, spray B) to contain a carboxylic acid with the same R_F as formic acid. The bulk of the distillate was treated with magnesium powder and N-hydrochloric acid, filtered, and warmed with 2,4-dinitrophenylhydrazine reagent [5 ml.; prepared by dissolving 2,4-dinitrophenylhydrazine (5 g.) in methanol (75 ml.), water (25 ml.), and sulphuric acid (10 ml.)]. The product was obtained by chromatography on alumina and recrystallisation from ethanol as yellow needles (23 mg.), m. p. and mixed m. p. with authentic formaldehyde 2,4-dinitrophenylhydrazone 147°. The i.r. and u.v. absorption spectra of the product and the authentic specimen were identical. The solution remaining from the periodate oxidation was treated with sulphur dioxide, hydrolysed with N-sulphuric acid, and then allowed to react with 2,4-dinitrophenylhydrazine reagent (10 ml.). Chromatography of the precipitate (54 mg.) on alumina, dichloromethane and chloroform being used as eluants, gave three components; the last to be eluted was the major product. It was identified as methylglyoxal bis-2,4-dinitrophenylhydrazone, by m. p. and mixed m. p. determination [298—300° (decomp.)], and by comparison of the i.r. and u.v. absorption spectra with those of authentic material.

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[24] Hesse and Bucking, *Annalen*, 1949, **563**, 31.