185. *The Chemistry of Fungi. Part VI. Rosenonolactone from* Trichothecium roseum *Link.*

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From the dried mycelium of the mould grown on a Czapek–Dox medium two crystalline lactones, rosenonolactone and rosonolactone, have been isolated of which the former is produced in larger amount. Rosenonolactone is a lævorotatory, unsaturated, ketonic lactone, in all probability having empirical formula $C_{20}H_{28}O_3$, which on hydrogenation yields dihydrorosenonolactone and on treatment with alkali is transformed into *iso*rosenonolactone which on hydrogenation yields dihydro*iso*rosenonolactone identical with the alkali transformation product from dihydrorosenonolactone. Ozonolysis of rosenonolactone gives rise to an acid and formaldehyde, indicating the presence of a vinyl group. Oxidation of sodium rosenonate with potassium permanganate yielded an acid, $C_{20}H_{30}O_5$, which on decomposition with alkali gave rise to a ketone, $C_{10}H_{18}O$, and a dibasic keto-acid, $C_{10}H_{14}O_5$. The ketone has been degraded to a ketonic acid, $C_{10}H_{18}O_3$, and to an acid, $C_{10}H_{18}O_4$.

In the course of studies on the antibiotic activity of fungi, a vigorous specimen of *Trichothecium roseum* Link was isolated in pure culture from decaying apples. This organism, which is the cause of " pink rot " in the fruit and was identified for us by Professor Joh. Westerdijk of Baarn, is known to exhibit considerable antagonistic activity towards other fungi (*e.g.*, Whetzel, *Ont. Nat. Sci. Bull.*, 1909, **5**, 3; Brian and Hemming, *J. Gen. Microbiol.*, 1947, **1**, 158), and an examination of its growth under a variety of conditions and of the metabolic products formed seemed to be of considerable interest. As a result it has been found, *inter alia*, that the mycelium of this organism grown on a simple Czapek–Dox medium contains in addition to mannitol two new water-insoluble lactonic substances, m. p. 214° and m. p. 186°, respectively, of which the former is produced in a considerably larger yield than the latter. In view of the recent communication by Freeman and Morrison (*Nature*, 1948, **162**, 30) announcing the isolation of the specific antifungal principle from the metabolic liquor of *T. roseum* Link, grown on a modified Czapek–Dox medium, we wish to record the results of our studies on the chemistry of the chief mycelial constituent, m. p. 214°, isolated in this laboratory in 1946.

The lactone, m. p. 214°, has been found to be a lævorotatory, unsaturated ketonic compound for which the analytical results, together with those of its derivatives and transformation products, indicate that the compound, for which we propose the name *rosenonolactone*, has in all probability the empirical formula $C_{20}H_{28}O_3$, although the formula $C_{19}H_{26}O_3$ is not entirely excluded. For the present we have adopted the former C_{20} formula for the lactone, which readily forms an *oxime*, a semicarbazone, and a 2 : 4-*dinitrophenylhydrazone*. On hydrogenation with hydrogen and a palladium–charcoal catalyst, the substance rapidly absorbed one molecule of hydrogen, giving rise to a dihydro-derivative, *dihydrorosenonolactone*, $C_{20}H_{30}O_3$, which like the parent compound retains the ketonic group and yields an *oxime*, a 2 : 4-*dinitrophenylhydrazone*, and a semicarbazone. That the reactive ethylenic linkage in rosenonolactone is present in a vinyl group is shown by the fact that on ozonolysis the compound gives rise to formaldehyde and a carboxylic *acid*, $C_{18}H_{25}O_3(CO_2H)$. In the course of experiments designed to test for the presence of a lactone group in rosenonolactone, which is insoluble in aqueous sodium carbonate and in cold aqueous sodium hydroxide, it was found that, on being heated with aqueous alcoholic sodium or potassium hydroxide, the compound dissolves, forming a solution which remains clear on dilution with water before or after evaporation of the alcohol and on being kept does not deposit solid. Acidification of this solution with mineral acid gives an almost theoretical yield of the isomeric lactone, iso*rosenonolactone*, $C_{20}H_{28}O_3$, in which the keto-group is intact since the compound readily forms an *oxime* and also a 2 : 4-dinitrophenylhydrazone apparently identical with the corresponding derivatives obtained under the same conditions from the parent lactone. When the hydrolytic isomerisation is effected with a known amount of the reagent, the titration of the excess with standard acid and phenolphthalein as an indicator shows that *iso*rosenonic acid is present as a monosodium salt. By conversion of this salt into the corresponding silver salt and treatment of the latter with methyl iodide in boiling acetone, *methyl* iso*rosenonate* was obtained. *iso*Rosenonolactone retains the ethylenic bond present in the parent lactone, and on hydrogenation gives rise to a dextrorotatory dihydro-derivative, *dihydro*iso*rosenonolactone*, $C_{20}H_{30}O_3$, identical with the compound formed by the action of boiling aqueous-alcoholic sodium hydroxide on dihydrorosenonolactone, clearly showing that the ethylenic linkage is not concerned in or necessary to the conversion of rosenonolactone into its isomeride. Prepared by either route, dihydro*iso*rosenonolactone gave the same *oxime* and 2 : 4-dinitrophenylhydrazone and was converted into *methyl dihydro*iso*rosenonate* by way of the silver salt. Contrary to expectation, prolonged action of acetic anhydride–pyridine on *iso*rosenonolactone gave a product which appears to be a *monoacetyl* derivative and which on treatment with a warm aqueous-alcoholic sulphuric acid solution of 2 : 4-dinitrophenylhydrazine sulphate was deacetylated giving rise to the 2 : 4-dinitrophenylhydrazone of *iso*rosenonolactone. Similarly, dihydroisorosenonolactone yielded a *monoacetyl* derivative. In both cases these derivatives are in all probability obtained from the enolic form of the respective ketones.

On being boiled with alcoholic hydrochloric acid, rosenonolactone gives rise to two isomeric compounds (A) and (B) in addition to *iso*rosenonolactone, a reaction which presumably proceeds by way of the latter substance since under the same conditions it gives (A) and (B) along with some unchanged material. In both cases (A), which is a neutral ketonic lactone and which we have named allo*rosenonolactone*, is the main product of the reaction and only small amounts of (B) have been obtained. Unlike *allo*rosenonolactone, the latter substance is readily soluble in aqueous sodium hydroxide or sodium carbonate but does not appear to react with sodium hydrogen carbonate; so far, however, we have no evidence that the compound is phenolic. Interaction of *allo*rosenonolactone with warm alcoholic 2 : 4-dinitrophenylhydrazine reagent gives rise to a 2 : 4-dinitrophenylhydrazone identical with the compound formed from rosenono- and *iso*rosenono-lactone under the same conditions. It seems reasonably certain, therefore, that in the acid medium employed in the preparation of the 2 : 4-dinitrophenylhydrazone rosenono- and *iso*rosenono-lactone undergo isomerisation to *allo*rosenonolactone which then reacts to give the hydrazone.

When rosenonolactone, *iso*rosenonolactone, or either of the dihydro-compounds was boiled with 50% alcoholic potassium hydroxide or fused with potassium hydroxide, traces of a volatile ketonic neutral compound were obtained which had a characteristic menthone-like odour. Similarly, oxidation of rosenono- or of *iso*rosenono-lactone gave rise to traces of the same steam-volatile odoriferous compound, which was finally obtained in quantity by the following route. Oxidation of sodium dihydro*iso*rosenonate with potassium permanganate in aqueous sodium carbonate gives rise to an *acid*, $C_{20}H_{30}O_5$, which appears to contain the carbon skeleton intact. Though we are satisfied that this acid retains at least one carbonyl group, we have not yet succeeded in isolating a stable functional ketonic derivative. On attempting to acetylate this compound by the pyridine method at room temperature, an *anhydro*-derivative, $C_{20}H_{28}O_4$, was obtained, which retains the carboxyl group of the parent substance intact, being readily soluble in aqueous sodium hydrogen carbonate. Although the parent acid appears to be reasonably stable to warm alcoholic sulphuric acid, it is gradually decomposed with warm, more slowly with cold, aqueous sodium hydroxide, giving rise to the required ketone and an *acid*. The latter compound, which forms a 2 : 4-dinitrophenylhydrazone, appears to be a dibasic acid, probably $C_{10}H_{14}O_5$, titrating potentiometrically with only one inflexion in the curve, a result which would seem to indicate that the carboxyl groups may be symmetrically placed in the molecule and do not interfere with each other. From its composition and the absence of a double bond it appears that this acid is a cyclic compound, but a study of its constitution is reserved for a later communication. With diazomethane the compound formed a liquid neutral dimethyl ester which was readily characterised by the formation of a *semicarbazone*.

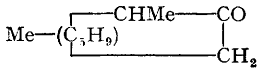
Unlike the parent acid $C_{20}H_{30}O_5$, the anhydro-compound appears to be stable to warm aqueous sodium hydroxide and does not give the ketone $C_{10}H_{18}O$.

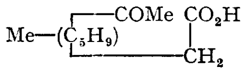
The ketonic substance $C_{10}H_{18}O$, which is isolated as a volatile oil, forms a well-crystallised *oxime* and a *semicarbazone*, the analytical results of which clearly indicate that the parent ketone has the empirical formula $C_{10}H_{18}O$. From *C*-methyl estimations on the semicarbazone, the compound probably contains at least two *C*-methyl groups and, since it appears to be saturated, it is clearly a cyclic ketone, as is suggested by its smell. In attempts to obtain further information regarding the structure of this compound, the oxime was submitted to the Beckmann rearrangement but the exact nature of the *product* has not yet been defined. Oxidation of the ketone with selenium dioxide in boiling acetic acid (but not in xylene) gave rise to a product from which a small amount of an *acid* was obtained, probably having the empirical formula $C_{10}H_{18}O_4$ rather than $C_{10}H_{16}O_4$ or $C_{10}H_{14}O_4$, a result which would seem to indicate that the oxidation has effected the opening of a ring system containing the keto-group. In this connection it may be noted that this acid gave a fluorescein reaction more closely resembling in this respect the behaviour of αβ-dimethylsuccinic acid rather than glutaric or adipic acid. On the other hand, prolonged treatment of the ketone with ozone and oxygen according to the procedure employed for *cyclo*hexanone by Stoll and Scherrer (*Helv. Chim. Acta*, 1930, **13**, 142) gave rise to an oily keto-acid which gave an iodoform reaction and was characterised by the formation of a *semicarbazone*, the analysis of which indicated that the parent keto-acid had the formula $C_{10}H_{18}O_3$.

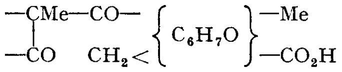
The transformations of rosenonolactone and its derivatives may be summarised as follows :

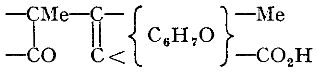
$$\begin{tikzcd}
\text{Rosenonolactone } C_{20}H_{28}O_3 \arrow[r, "H_2"] \arrow[d] & \text{Dihydrorosenonolactone } C_{20}H_{30}O_3 \arrow[d] & \\
\textit{iso}\text{Rosenonolactone } C_{20}H_{28}O_3 \arrow[r, "H_2"] \arrow[d] & \text{Dihydro}\textit{iso}\text{rosenonolactone } C_{20}H_{30}O_3 \arrow[d] & \\
\textit{allo}\text{Rosenonolactone } C_{20}H_{28}O_3 \text{ and Compound (B) } C_{20}H_{28}O_3 & \text{Acid } C_{20}H_{30}O_5 \arrow[d] \arrow[dr] & \\
\text{Keto-acid } C_{10}H_{18}O_3 & \text{Ketone } C_{10}H_{18}O \arrow[l] & \text{Keto-dibasic acid } C_{10}H_{14}O_5
\end{tikzcd}$$

The oxidation of the cyclic ketone $C_{10}H_{18}O$ to a keto-acid $C_{10}H_{18}O_3$, giving an iodoform reaction, suggests that the parent compound may well be of the type (I) giving the oxidation product type (II). Although definite proposals regarding the mode of linking of the C_{10} residues in the acid $C_{20}H_{30}O_5$ must await the definition of the structures of these units, it would seem probable that the C_{10} residue, appearing as the keto-dibasic acid $C_{10}H_{14}O_5$ and originally

(I.)

$$\mathrm{Me{-}(C_5H_9)}\left<\begin{array}{l}\mathrm{CHMe{-}CO}\\ \qquad\quad\;\;|\\ \qquad\quad\mathrm{CH_2}\end{array}\right.$$


$$\mathrm{Me{-}(C_5H_9)}\left<\begin{array}{l}\mathrm{COMe\;\;CO_2H}\\ \qquad\quad\;\;|\\ \qquad\quad\mathrm{CH_2}\end{array}\right.$$
(II.)

(III.)

$$\begin{array}{l}\mathrm{-CMe-CO-}\\ \;\;|\\ \mathrm{-CO\quad CH_2<}\end{array}\left\{\mathrm{C_6H_7O}\right\}\begin{array}{l}\mathrm{-Me}\\ \\ \mathrm{-CO_2H}\end{array}$$


$$\begin{array}{l}\mathrm{-CMe-C-}\\ \;\;|\qquad\;\,\|\\ \mathrm{-CO\quad\;\; C<}\end{array}\left\{\mathrm{C_6H_7O}\right\}\begin{array}{l}\mathrm{-Me}\\ \\ \mathrm{-CO_2H}\end{array}$$
(IV.)

carrying the lactone system of dihydro*iso*rosenonolactone, must contain either an unreactive double bond or a dicyclic system. Further, the scission of the acid $C_{20}H_{30}O_5$ by alkalis could arise from the kind of system expressed in (III), and the dehydration of (III) to give an anhydro-compound no longer affected by warm dilute alkalis might be due to the condensation of a keto-group with an active methylene group as in (IV). In view of the fact that, like dihydro*iso*rosenonolactone, the parent lactone and its isomeride give rise to the ketone $C_{10}H_{18}O$ on degradation, it is clear that the double bond and consequently the vinyl group in rosenonolactone are situated in the residue giving the keto-dibasic acid $C_{10}H_{14}O_5$.

From the waxy residue left after the separation of rosenonolactone from the crude mycelial extract, a small amount of a second lactonic, slightly lævorotatory compound has been obtained, m. p. 186°, which does not appear to contain a reactive double bond or a reactive carbonyl group. For this compound, which appears to have empirical formula $C_{20}H_{30}O_3$, we propose the name *rosonolactone*, but the quantity of material available so far is insufficient for studies on its degradation.

EXPERIMENTAL.

Rosenonolactone.—A culture of *Trichothecium roseum* Link, syn. *Cephalothecium roseum* Corda, was isolated from a decaying apple in 1946 and stocks have been maintained on beer-wort agar slopes. In the present investigation, the cultivation of this mould, the growth of which is stimulated by the presence of corn-steep liquor or extract of peas in the medium, was studied with a view to producing optimum yields of the two crystalline metabolic products which had been isolated from the dried mycelium in the course of preliminary experiments. It was ultimately decided that the most satisfactory yields were obtained by employing the simple Czapek–Dox medium (pH 4·2) composed of sodium nitrate (2 g.), potassium chloride (0·5 g.), magnesium sulphate (0·5 g. of heptahydrate), ferrous sulphate (0·01 g. of heptahydrate), potassium dihydrogen phosphate (1 g.), and glucose (30 g.) in tap-water (1 l.) and inoculating with an aqueous suspension of spores produced on beer-wort agar. A thin mat of mycelium was grown on a shallow layer of medium (150 c.c. over an area of about 25 sq. cm.) at room temperature (15—18°) during 14 days, fresh sterile medium was added to make up the volume to approximately 1 l., and then growth was allowed to proceed at room temperature for a further 3 weeks. The faintly pink mycelium, coloured salmon-pink on the lower side, was collected, washed, dried, powdered, and extracted in a Soxhlet apparatus with ether; the yield of dried mycelium per l. of medium was 5·8—6·5 g., from which approximately 0·217 g. of the rosenonolactone was obtained. After having been exhaustively extracted with ether, a sample of the mycelium was extracted with acetone and on evaporation of the solvent mannitol was obtained, forming needles, m. p. 166°, from aqueous alcohol or acetone, identical with an authentic sample. The hexa-acetate had m. p. and mixed m. p. 122°. Subsequently it was found that the mould could be cultivated directly on deeper layers of medium.

The product (mixed with fatty material) obtained by evaporation of the ethereal extracts was warmed with alcohol, and the insoluble rosenonolactone collected. Recrystallised from benzene and then alcohol or from benzene only, this compound formed colourless squat prisms, m. p. 214°, $[\alpha]_D^{20^\circ}$ $-107·5°$ (*c*, 1·2 in chloroform), moderately soluble in cold ethyl acetate, chloroform, or acetone, sparingly soluble in cold alcohol, benzene, ether, and boiling light petroleum, and insoluble in water [Found: C, 75·8; H, 8·6; *M* (Rast), 361·3. $C_{20}H_{28}O_3$ requires C, 75·9; H, 8·9%; *M*, 316·4]. The 2 : 4-*dinitrophenylhydrazone* formed orange-yellow needles, m. p. 236° (decomp.), from alcohol (Found: C, 62·8; H, 6·0; N, 11·7. $C_{26}H_{32}O_6N_4$ requires C, 62·9; H, 6·5; N, 11·3%). Prepared by the pyridine method at room temperature or by the sodium acetate method in boiling alcohol for 2 hours, the *oxime* separated from alcohol in colourless elongated prisms, m. p. 212°, which on admixture with original compound had m. p. 165—185° (Found: N, 4·4. $C_{20}H_{29}O_3N$ requires N, 4·2%).

Rosonolactone.—The residues from the solutions left on the separation of the crude rosenonolactone fraction, consisting mainly of waxy and fatty material, were triturated with methanol which dissolves the fat and waxy product, leaving *rosonolactone* together with small amounts of rosenonolactone. This residue was extracted with boiling methanol or 90% alcohol, and the extract filtered from a little undissolved rosenonolactone. The solid which was obtained by evaporation of the filtrate was repeatedly treated in the same manner until all the rosenonolactone had been removed. Finally, rosonolactone was crystallised from 90% alcohol or from light petroleum, forming colourless rod-like prisms, m. p. 186°, $[\alpha]_D^{20^\circ}$ $-3·0°$ (*c*, 1 in chloroform) (Found: C, 75·8, 75·7, 75·9; H, 9·3, 9·5, 9·6. $C_{20}H_{30}O_3$ requires C, 75·4; H, 9·5%). This compound is considerably more soluble in the usual organic solvents than rosenonolactone. On being boiled with aqueous-alcoholic sodium hydroxide the lactone dissolves, and on evaporation of the alcohol and dilution of the residue with water a clear solution is obtained which only deposits the lactone after having been acidified with hydrochloric acid.

Dihydrorosenonolactone.—Hydrogenation of rosenonolactone (5 g.), dissolved in ethyl acetate (800 ml.), with hydrogen at atmospheric pressure and a palladium–charcoal catalyst (from 1·5 g. of charcoal and 0·15 g. of palladium chloride) was complete in 30—40 minutes. Evaporation of the filtered solution gave the *dihydro*-compound (4·9 g.), m. p. 181—182°, which on recrystallisation from alcohol formed elongated prisms, rather more soluble than the parent substance in the usual organic solvents, m. p. 183°, $[\alpha]_D^{20^\circ}$ $-106·0°$ (*c*, 1 in chloroform) (Found: C, 75·4; H, 9·3. $C_{20}H_{30}O_3$ requires C, 75·4; H, 9·5%). Estimation of *C*-methyl gave 11·4%, whereas $C_{18}H_{24}O_3Me_2$ requires 9·4% and $C_{17}H_{21}O_3Me_3$ requires 14·1%. The *oxime* separated from dilute alcohol in colourless needles, m. p. 250° (decomp.) (Found: N, 4·5. $C_{20}H_{31}O_3N$ requires N, 4·2%), and the semicarbazone from the same solvent in colourless prisms, m. p. 205—207°. The 2 : 4-*dinitrophenylhydrazone* formed tiny yellow needles, m. p. 250° (decomp.), from alcohol–benzene (Found: N, 11·7. $C_{26}H_{34}O_6N_4$ requires N, 11·3%).

Action of Alcoholic Sodium Hydroxide on Rosenonolactone.—When the lactone (5 g.) was boiled with 5% alcoholic sodium hydroxide (50 ml.) for ½ hour, and the solution cooled and acidified with dilute hydrochloric acid, iso*rosenonolactone* was obtained in colourless, rod-like prisms (4·9 g.), m. p. 140—142°, which on recrystallisation from dilute alcohol had m. p. 144°, $[\alpha]_D^{20^\circ}$ $+20·0°$ (*c*, 1·5 in chloroform) [Found: C, 75·9; H, 8·8; *M* (Rast), 325. $C_{20}H_{28}O_3$ requires C, 75·9; H, 8·9%; *M*, 316·4]. When the hydrolysis is conducted with a measured excess of alcoholic alkali and the excess titrated with phenolphthalein as an indicator, after the completion of the reaction the results indicated that 1 equiv. of sodium hydroxide is required in the formation of the sodium salt of the acid.

For the preparation of the methyl ester of the acid from *iso*rosenonolactone, the compound (1 g.) was heated under reflux with 0·5N-alcoholic sodium hydroxide (7 ml.) for ½ hour, the alcohol was distilled off, the residue was treated with water (150 ml.), the cooled solution was filtered to remove traces of unchanged *iso*rosenonolactone, and the liquor treated drop-wise with silver nitrate solution (0·7 g. in 5 ml. of water). The resulting insoluble silver salt was collected, washed with methanol, and boiled with acetone

(100 ml.) and methyl iodide (2 ml.) for 1 hour. Evaporation of the filtered solution and treatment of the residue with water precipitated the *methyl* ester, which formed colourless needles, m. p. 157—158°, from dilute methanol [Found : C, 72·2, H, 9·7; OMe, 7·0 $C_{20}H_{29}O_3(OMe)$ requires C, 72·4; H, 9·3; OMe, 9·0%].

Interaction of *iso*rosenonolactone with excess of hydroxylamine hydrochloride and pyridine at room temperature for 3 days gave the *oxime*, which separated from aqueous alcohol in elongated colourless prisms, m. p. 180—181° (Found : N, 4·5. $C_{20}H_{29}O_3N$ requires N, 4·2%). The 2 : 4-*dinitrophenylhydrazone* formed yellow elongated prisms, m. p. 231° (decomp.), from 90% alcohol (Found : N, 11·8. $C_{26}H_{32}O_6N_4$ requires N, 11·3%).

Interaction of *iso*rosenonolactone (0·4 g.), acetic anhydride (2 ml.), and pyridine (2 ml.) at room temperature during 4 days gave an *acetyl* derivative (0·4 g.), forming colourless needles, m. p. 183°, from dilute alcohol, $[\alpha]_D^{20°}$ −47·0° (*c*, 1 in chloroform) (Found : C, 73·7; H, 8·4; CH_3·CO, 12·9. $C_{22}H_{30}O_4$ requires C, 73·7; H, 8·4; CH_3·CO, 12·0%). Interaction of this compound in alcohol with a warm dilute sulphuric acid solution of 2 : 4-dinitrophenylhydrazine sulphate gave rise to the 2 : 4-dinitrophenylhydrazone of *iso*rosenonolactone, m. p. 231° (decomp.) after purification.

Action of Alcoholic Hydrochloric Acid on Rosenono- and iso*Rosenono-lactone.*—A solution of rosenonolactone (0·5 g.) in N-alcoholic hydrochloric acid (50 ml., prepared by diluting concentrated acid with alcohol) was heated on the steam-bath for 2 hours, and on being basified with 2N-aqueous sodium hydroxide the cooled reaction mixture deposited crystalline material. This product was fractionally crystallised from alcohol, giving three main crops : (1) slightly impure rosenonolactone, m. p. 205—206°; (2) *allorosenonolactone* (A), which formed prisms (0·1 g.), m. p. 216°, after repeated recrystallisation from alcohol and on admixture with rosenonolactone had m. p. 204—206° [Found : C, 75·9; H, 8·8; *M* (Rast), 316·5. $C_{20}H_{28}O_3$ requires C, 75·9; H, 8·9%; *M*, 316·4]; (3) *iso*rosenonolactone, m. p. and mixed m. p. 144°, from the alcoholic residues left after the separation of the crude *allo*rosenonolactone.

Acidification of the aqueous liquor left on filtration of the crystalline product with carbon dioxide gave a solid (B) in needles (50 mg.), m. p. 186—190°, which on recrystallisation from benzene or aqueous acetone had m. p. 210° and was soluble in dilute aqueous sodium hydroxide or carbonate but not in aqueous sodium hydrogen carbonate (Found : C, 75·8; H, 8·7. $C_{20}H_{28}O_3$ requires C, 75·9; H, 8·9%).

Under the same conditions *iso*rosenono- gave *allo*rosenonolactone, m. p. and mixed m. p. 216°, and (B), m. p. and mixed m. p. 210° after purification, along with unchanged *iso*rosenonolactone.

Ozonolysis of Rosenonolactone.—A slow stream of ozone and oxygen was passed into a solution of the lactone (0·5 g.) in anhydrous chloroform (50 ml.) for 1 hour, and the solution evaporated in a vacuum. The combined residual products from two experiments were treated with water (200 ml.), and next day the mixture was heated on the steam-bath for 45 minutes, part of the aqueous liquor was distilled off, and the distillate (50 ml.) treated with an aqueous solution of 2 : 4-dinitrophenylhydrazine sulphate, giving a precipitate of formaldehyde 2 : 4-dinitrophenylhydrazone, m. p. 163—164°, identical with an authentic specimen. The residual aqueous liquor in the distilling flask was repeatedly extracted with ether, and the combined extracts washed with dilute aqueous sodium hydroxide. Acidification of the alkaline washings gave an *acid*, which separated from alcohol or aqueous acetone in colourless needles (0·15 g.), m. p. 260° (decomp.) [Found : C, 68·4; H, 7·9; *M* (by titration), 310. $C_{19}H_{26}O_5$ requires C, 68·3; H, 7·8%; *M*, 334]. The oxime of this acid formed prisms, m. p. 256—257°, from dilute alcohol. Mixed with the parent substance this derivative melted at 230—240°.

*Dihydro*iso*rosenonolactone.*—Treatment of dihydrorosenonolactone with warm dilute alcoholic sodium hydroxide, according to the procedure employed for converting rosenono- into *iso*rosenono-lactone, gave rise to *dihydro*iso*rosenonolactone*, which formed colourless rod-like prisms, m. p. 150°, $[\alpha]_D^{20°}$ +34·0° (*c*, 1 in chloroform), from dilute alcohol, easily soluble in alcohol (Found : C, 75·4; H, 9·4. $C_{20}H_{30}O_3$ requires C, 75·4; H, 9·5%). The 2 : 4-dinitrophenylhydrazone formed tiny elongated prisms, m. p. 250° and mixed m. p. 250° (decomp.), identical with the derivative from dihydrorosenonolactone (Found : N, 11·7%). Acetylation of the lactone with acetic anhydride–pyridine at room temperature for 4 days gave the *acetyl* derivative, which separated from alcohol in colourless elongated prisms, m. p. 184°, $[\alpha]_D^{20°}$ −34·0° (*c*, 1 in chloroform) [Found : C, 73·6; H, 8·9; CH_3·CO, 12·7. $C_{20}H_{29}O_3(CO{\cdot}CH_3)$ requires C, 73·3; H, 9·0; CH_3·CO, 12·0%]. Prepared by way of the sodium salt and the silver salt, the *methyl* ester of dihydro*iso*rosenonic acid formed colourless shining plates, m. p. 165°, from dilute methanol [Found : C, 72·1; H, 9·3; OMe, 6·7. $C_{20}H_{31}O_3(OMe)$ requires C, 72·0; H, 9·7; OMe, 8·9%]. Hydrogenation of *iso*rosenonolactone (2 g.), dissolved in alcohol (250 ml.), with hydrogen at atmospheric pressure and a palladium-charcoal catalyst (from 1 g. of charcoal and 0·1 g. of palladium chloride) was complete in about 30 minutes and on isolation the *dihydro*-derivative (1·9 g.), m. p. 147°, separated from dilute alcohol in colourless needles, m. p. 150°, identical with a specimen prepared by the action of alkali on dihydrorosenonolactone (Found : C, 75·3; H, 9·2%). The acetyl derivative had m. p. and mixed m. p. 184°.

*Oxidation of Dihydro*iso*rosenonolactone.*—Hydrogenation of rosenonolactone (10 g.) gave the dihydrolactone (9·8 g.), which was heated under reflux with 0·5N-alcoholic sodium hydroxide (83 ml., *i.e.*, 8·5 ml. per g.) for ½ hour. After having been diluted with water, the alcohol was removed completely by distillation in a vacuum, and the volume of the residue made up to one litre with water. Next day the solution was filtered to remove a small amount of unchanged material and divided into two equal portions. Each portion was shaken and treated with a mixture of equal parts of powdered potassium permanganate and anhydrous sodium carbonate added in portions of 0·1—0·2 g. At first the permanganate was taken up rapidly, but after 3—4 hours, when 2·8 g. of permanganate had been added, the oxidation proceeded much more slowly. At this stage the combined solutions were decolourised with the minimum amount of sulphur dioxide, acidified with hydrochloric acid, and kept for 24 hours. The white crystalline precipitate was collected and extracted with a little aqueous sodium carbonate , leaving unchanged dihydro*iso*rosenonolactone (4·22 g.), and on acidification the alkaline extract gave acidic material (4·4 g.). The unchanged dihydro*iso*rosenonolactone was converted into the sodium salt of the acid and oxidised with proportionate amounts of potassium permanganate. Eventually a combined yield of 6·28 g. of acidic product together with 2·05 g. of unchanged lactone was obtained. The acidic fraction, which contained much gummy impurities, had m. p. 180—200° and, on crystallisation from ethyl acetate and then acetone, formed small colourless needles, m. p. 232°, $[\alpha]_D^{20°}$ +4·0° (c, 1 in chloroform) (Found : C, 68·3; H, 8·5; *M* (by titration), 386. $C_{20}H_{30}O_5$ requires C, 68·6; H, 8·6%; *M*, 350·4). This compound, which is only slightly soluble in hot water and readily soluble in aqueous sodium hydrogen carbonate, does not appear to react readily with semicarbazide acetate or with 2 : 4-dinitrophenylhydrazine. On treatment with ethereal diazomethane the acid gave a *methyl* ester, forming prisms, m. p. 97—98° [Found : OMe, 8·5. $C_{20}H_{29}O_4(OMe)$ requires OMe, 8·5%], from dilute methanol. Attempted acetylation by the pyridine method at room temperature gave an *anhydro*-derivative which was acidic, being readily soluble in aqueous sodium hydrogen carbonate, and formed colourless blunt needles, m. p. 212°, from dilute acetone (Found : C, 72·6; H, 8·5. $C_{20}H_{28}O_4$ requires C, 72·3; H, 8·5%). Unlike the parent acid, this compound does not appear readily to form the cyclic ketone responsible for the menthone-like odour.

A solution of the acid (1 g.), m. p. 232°, in 0·5N-aqueous sodium hydroxide (40 ml.) was kept at room temperature for 3 days; after a few hours the pale yellow liquid developed the characteristic odour, and oily globules began to separate. The solution was diluted somewhat, and the volatile ketonic product removed by a current of steam and then isolated from the distillate with ether. The aqueous residual hydrolysate was acidified with dilute hydrochloric acid and extracted three times with ether. Evaporation of the combined extracts left a small amount of acid which has not yet been investigated. The acidified aqueous liquors were then repeatedly extracted with ethyl acetate, and on evaporation of the dried extracts a pale yellow crystalline *acid* (0·35—0·4 g.), m. p. 199—200°, was obtained. Recrystallised from ethyl acetate, this compound formed large colourless polyhedra, m. p. 205° after softening at 195°, $[\alpha]_D^{20°}$ −28·0° (*c*, 1 in water), insoluble in cold ether, benzene, or chloroform, sparingly soluble in cold ethyl acetate or acetone, and moderately soluble in water or dioxan (Found : C, 56·1; H, 6·4. $C_{10}H_{14}O_5$ requires C, 56·1; H, 6·5%). The equivalent weight of this compound as determined by titration with alkali was 87·6, but by potentiometric titration the value was 110, whereas the theoretical equivalent for two carboxyl groups is 107. In the latter titration only one inflexion point was obtained on the curve. The 2 : 4-dinitrophenylhydrazone formed yellow needles, m. p. 205—206°, from benzene-alcohol. Esterification of this acid with ethereal diazomethane yielded a liquid dimethyl ester which gave rise to a *semicarbazone*, forming elongated prisms, m. p. 217—218°, from benzene-methanol [Found : OMe, 19·7; N, 13·6. $C_{11}H_{15}O_3N_3(OMe)_2$ requires OMe, 20·7; N, 14·0%].

The Ketone $C_{10}H_{18}O$.—This compound was isolated from the foregoing oxidation product as a colourless oil (0·35 g.) which readily yielded ketonic derivatives but did not decolourise neutral potassium permanganate in acetone. The crude ketone (1·07 g.) was readily oximated with excess of hydroxylamine hydrochloride and sodium acetate, and the *oxime* was separated from the reaction mixture by distillation in a current of steam, crystallising from the distillate in elongated prisms. Recrystallised from dilute methanol, the compound (0·92 g.) had m. p. 56—57° (Found : C, 70·3; H, 11·2; N, 8·1; *C*-Me, 16·7. $C_8H_{13}ONMe_2$ requires C, 71·0; H, 11·3; N, 8·3; *C*-Me, 17·8%). Prepared in the usual way, the *semicarbazone* separated from methanol or alcohol in colourless needles, m. p. 178°, $[\alpha]_D^{20°}$ +17·0° (*c*, 1 in chloroform) (Found : C, 62·3; H, 9·7; N, 20·0. $C_{11}H_{21}ON_3$ requires C, 62·6; H, 10·0; N, 19·9%).

Thionyl chloride (0·5 ml.) in ether (5 ml.) was added drop-wise to a solution of the oxime (0·48 g.) in ether (20 ml.) kept at about −5° during 10 minutes. The white precipitate which first separated became slightly gummy. After having been kept for a further 10 minutes, the reaction mixture was treated with water (25 ml.) and extracted with ether. Evaporation of the dried extracts left an oil which rapidly solidified, m. p. 50—52°, and on recrystallisation from light petroleum (b. p. 40—45°) and then water, this *compound* formed colourless needles, m. p. 55°, which on admixture with the oxime melted at room temperature (Found : C, 64·4; H, 11·4; N, 7·5. $C_{10}H_{19}ON,H_2O$ requires C, 64·2; H, 11·2; N, 7·5%. Calc. for $C_{10}H_{19}ON$: C, 71·0; H, 11·2; N, 8·3%). The substance is volatile in steam, and on being dried in a vacuum desiccator melts and then resolidifies on exposure to the moist atmosphere.

On being heated, a mixture of the ketone (1·2 g.), selenium dioxide (1·2 g.), and acetic anhydride (1·5 ml.) began to boil at 130° (oil-bath) and 5 minutes later the temperature was raised to 140°. After having been kept at 140° for 35 minutes, the cooled mixture was filtered, almost neutralised with sodium carbonate, and extracted with ether. Repeated extraction of the ethereal solution with aqueous sodium hydroxide followed by acidification of the combined alkaline extracts and then the removal of traces of volatile matter by a current of steam left a non-volatile residue which was isolated with ether and triturated with dilute hydrogen peroxide. Crystallised from benzene-light petroleum (b. p. 60—80°), the *acid* formed colourless prisms, m. p. 145—146°, insoluble in water but readily soluble in aqueous sodium hydrogen carbonate (Found : C, 59·8; H, 8·8. $C_{10}H_{18}O_4$ requires C, 59·4; H, 8·9%. $C_{10}H_{16}O_4$ requires C, 60·0; H, 8·0%).

A slow stream of ozone and oxygen, saturated with carbon tetrachloride vapour at room temperature, was passed into a solution of the ketone $C_{10}H_{18}O$ (0·9 g.) in carbon tetrachloride (5 ml.) at room temperature for 20 hours. The solution was diluted with ether and extracted with aqueous sodium carbonate. From the ether a mixture (0·52 g.) of unchanged ketone and another product was obtained, and acidification of the aqueous sodium carbonate extracts gave a liquid keto-acid (0·36 g.), which was isolated with ether and purified by distillation in a vacuum, giving two fractions : (*a*) (30 mg.), b. p. 80—100°/23 mm., and (*b*) (200 mg.), b. p. 180—210°/23 mm. The latter fraction, which gave the reactions of a ketonic acid and an iodoform reaction, was converted into the *semicarbazone* (0·08 g. from 0·11 g. of acid), which formed prisms, m. p. 166°, $[\alpha]_D^{20°}$ +12·0° (*c*, 0·5 in alcohol), from dilute alcohol (Found : C, 54·4; H, 8·7; N, 17·2. $C_{11}H_{21}O_3N_3$ requires C, 54·3; H, 8·6; N, 17·3%).

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