Studies in the Biochemistry of Micro-Organisms

88. PALITANTIN. PART 2. FURTHER DERIVATIVES AND DEGRADATION PRODUCTS

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(Received 19 October 1951)

The isolation of the mould metabolic product, palitantin, $C_{14}H_{22}O_4$, obtained from *Penicillium palitans* Westling, was described by Birkinshaw & Raistrick (1936). The product was characterized as an aldehyde containing two hydroxyl groups and apparently possessing two double bonds. The function of the fourth oxygen atom was not determined. Further derivatives and degradation products of palitantin have been prepared and are described in the present communication.

In the earlier paper (Birkinshaw & Raistrick, 1936) palitantin was described as optically inactive, since under the experimental conditions employed the rotation observed, $+0.01^\circ$, was regarded as zero within the limits of experimental error. Some of the derivatives and breakdown products have now been found to show considerable optical activity. When palitantin was re-examined under more favourable conditions it was found to have a rotation of $[\alpha]_{5461}^{23} + 4.4^\circ$ (c, 0.8 in $CHCl_3$).

In a quantitative study of the periodate oxidation of palitantin, tetrahydropalitantin, and tetrahydropalitantic acid, the following points have been established. (1) Palitantin and tetrahydropalitantin react with potassium periodate using the equivalent of 2 atoms of oxygen. (2) Two acidic groups are produced, one of which readily lactonizes. (3) An aldehyde group is also produced. (4) The aldehyde and lactonic groupings are constituents of a C_{13} molecule, the other carbon atom being detached as formic acid. (5) With tetrahydropalitantic acid, only one oxygen atom is used, no acidic group is produced but two carbonyl groups are formed.

These facts appear capable of a simple explanation, namely that the reactive grouping in palitantin is part of a ring, and has the form I.

This would be split at the dotted lines by 2 molecules of periodic acid to yield form II plus 1 molecule of formic acid. With tetrahydropalitantic acid,

in which the aldehyde group of tetrahydropalitantin has been oxidized to carboxyl, the starting material would be represented by form III which on oxidation with 1 molecule of periodic acid would pass to IV.

Since the C_{13} acid lactone from tetrahydropalitantin gives rise on nitric acid oxidation to a *n*-heptyl succinic acid, tetrahydropalitantin would appear to consist of a 6-carbon ring with two side chains, one being a CHO group and the other a *n*-heptyl chain. One double bond in palitantin must be situated $\gamma\delta$ to the terminal methyl group of the C_7 chain to account for the production of *n*-butyraldehyde on ozonolysis. The position of the second double bond, if present, is still obscure, likewise the function of the fourth oxygen atom which is presumably attached to a ring carbon atom.

The examination of palitantin continues and it is hoped to discuss its constitution more fully in a later paper.

The dextrorotatory acid which was obtained, together with palitantin, as a metabolic product of the type strain of *Penicillium palitans* (Birkinshaw & Raistrick, 1936, p. 803), has been further examined. It crystallizes from water in colourless needles, m.p. 134.5° , $[\alpha]_{5461}^{24} + 82^\circ$ (in $CHCl_3$). It has the empirical formula $C_{14}H_{20}O_4$, titrates as a monobasic acid and gives a reddish-purple colour with ferric chloride. Curtis, Hemming & Smith (1951) have recently isolated an antifungal metabolic product, frequentin, from a strain of *Penicillium frequentans* Westling, which was also found to produce palitantin. From the published description frequentin appeared to be very similar to the acid product from *P. palitans*. By a comparison of samples it has been established that the two products are in fact identical; there was no depression of melting point on mixing. The acidic product from *P. palitans* is

therefore frequentin. Curtis & Duncanson (1952) have now shown that frequentin and palitantin are closely related in structure.

EXPERIMENTAL

All melting points are uncorrected.

Properties of palitantin and its derivatives

Specific rotations. Birkinshaw & Raistrick (1936) stated that palitantin is optically inactive. This opinion was based on the fact that a solution of palitantin (0.1505 g.) in CHCl_3 (15 ml.) had an observed average rotation of $+0.01^\circ$ (limits of five readings 0.00° to $+0.02^\circ$) in a 10 cm. tube in the Hg green light (5461 Å.) at 20° . This corresponds to $[\alpha]_{5461}^{20} = +1.0^\circ$, which was considered to be within the limits of experimental error.

When it was subsequently observed that derivatives of palitantin have relatively high specific rotations, the specific rotation of palitantin was redetermined. A solution of palitantin in CHCl_3 was exhaustively extracted with aqueous alkali to remove traces of frequentin (see p. 276). The purified CHCl_3 solution was evaporated to dryness *in vacuo*, and the residual palitantin was crystallized a number of times from boiling water. A solution of the purified palitantin (0.3941 g.) in CHCl_3 (50 ml.) in a 40 cm. tube had an observed average rotation of $+0.14^\circ$ (limits of seven readings $+0.13^\circ$ to $+0.15^\circ$), corresponding to $[\alpha]_{5461}^{23} = +4.4^\circ$.

The observed specific rotations of palitantin and some of its derivatives are recorded in Table 1.

whole of the volatile acids had been removed in the distillate. The total volatile acid (10.23 ml. of 0.1 N) corresponds to 1.003 mol. of acetic acid/mol. of palitantin.

Tetrahydropalitantin. Tetrahydropalitantin (0.2876 g.) on similar oxidation yielded 11.03 ml. 0.1 N volatile acid, equivalent to 0.989 mol. of acetic acid per mol. of tetrahydropalitantin.

Reaction of palitantic acid with sodium amalgam and of palitantin with maleic anhydride

With sodium amalgam. Palitantic acid (1 g.) was treated with Na amalgam (2.5% Na; 100 g.) and water with vigorous stirring. The reaction was maintained about neutral by frequent additions of H_2SO_4 . The solution was filtered whilst slightly alkaline and acidified. Crystals (0.8 g.) were obtained, which proved to be unchanged palitantic acid. The reduction was therefore continued using a further 400 g. of amalgam over a period of 2 days. Unchanged palitantic acid (0.65 g.) was again recovered, an almost quantitative recovery when allowance is made for the solubility of palitantic acid. A further attempt to obtain reduction in acidic ethanol also failed. This renders unlikely the presence of a conjugated double bond or of a grouping capable of keto-enol tautomerism.

Action of maleic anhydride on palitantin. A solution of palitantin (0.2 g.) and maleic anhydride (0.1 g.) in benzene (100 ml.) was refluxed for 1.5 hr. On cooling, crystals separated and were collected, washed with benzene and dried, wt. 0.13 g., m.p. $164\text{--}165^\circ$ not depressed on admixture with palitantin. Hence palitantin does not appear to contain a conjugated double bond system.

Table 1. *Specific rotation of palitantin and its derivatives*

Substance	Temperature ($^\circ$)	Solvent	Vol. of solvent (ml.)	Wt. of substance (g.)	Length of tube (cm.)	$[\alpha]_{5461}^{20}$ ($^\circ$)
Palitantin	23	CHCl_3	50	0.3941	40	+ 4.4
Palitantin monophenyl- hydrazone	24	Pyridine	1	0.0088	5 microtube	- 93
Palitantin bis- <i>p</i> -bromo- benzoate	24	Ethanol	1	0.0110	5 microtube	- 13
Palitantic acid	20	Ethanol	1	0.0100	5 microtube	- 74
α -Hexahydropalitantin	24	Ethanol	1	0.0074	5 microtube	- 54
β -Hexahydropalitantin	24	Ethanol	1	0.0108	5 microtube	- 72
Tetrahydropalitantic acid	24	Ethanol	1	0.0102	5 microtube	- 90
Tetrahydropalitantin bis- <i>p</i> -bromobenzoate	23	Ethanol	25	0.0666	20	- 2
Tetrahydropalitantin	24	Ethanol	50	0.5836	40	- 43.1

Carbon-methyl groups

Palitantin. 1.19 and 1.18 mol. of acetic acid were produced per mol. of palitantin in the Kuhn-Roth micromethod. (Estimations by Dr Roth.)

A mixture of palitantin (0.2589 g.), CrO_3 (12 g.), $\text{K}_2\text{Cr}_2\text{O}_7$ (3 g.), water (30 ml.) and H_3PO_4 (sp.gr. 1.75, 20 ml.) was heated on a boiling-water bath under reflux for 3.5 hr. (Karrer, Helfenstein, Wehrli & Wettstein, 1930). A distinct smell of butyric acid was observed. The oxidation mixture, contained in a Claisen flask, was distilled *in vacuo* until the

Derivatives of palitantin, tetrahydropalitantin and palitantic acid

Monoiodopalitantin. 0.1 N Aqueous I_2 solution (100 ml.) was added to a solution of palitantin (0.5 g.) in ethanol (25 ml.). The solution, from which crystals soon began to separate, was held for 1 hr. initially at room temperature and finally in the refrigerator. The excess I_2 was removed by titration with chilled 0.1 N- $\text{Na}_2\text{S}_2\text{O}_3$. I_2 , equivalent to 40 ml. 0.1 N, was absorbed, corresponding almost quantitatively to

the uptake of 2 atoms of I/mol. of palitantin. The slightly yellow crystals (0.47 g.) were collected, dried and recrystallized from aqueous ethanol (2 vol. water: 1 vol. ethanol). Beautiful long colourless silky needles (0.30 g.) of *monoiopalitantin* separated, m.p. 136° (sharp, but decomposing to a black liquid which froths up the melting point tube). (Found: C, 44.5, 44.6; H, 5.7, 5.7; I, 33.7, 33.4. $C_{14}H_{21}O_4I$ requires: C, 44.2; H, 5.6; I, 33.4%.) Monoiopalitantin is thus derived according to the equation

Monoiopalitantic acid. 0.1N Aqueous I_2 solution (20.0 ml.) was added to a solution of palitantic acid (0.0540 g.) in 0.1N aqueous NaOH (2.05 ml.), and the whole made up to a total volume of 50.0 ml. After 1 hr. the residual I_2 was estimated by titration with 0.1N- $Na_2S_2O_3$. Iodine equivalent to 1.95 atoms/mol. of palitantic acid was absorbed. The titrated solution was acidified with 0.1N-HCl (2 ml.). After chilling, colourless needles (0.023 g.) of *monoiopalitantic acid* separated and were collected, washed and dried. Melting point 135.5°, darkening on melting, but not blackening or frothing as did monoiopalitantin. (Found: C, 42.1, 42.3; H, 5.5, 5.6; I, 31.8, 31.5. $C_{14}H_{21}O_5I$ requires: C, 42.4; H, 5.3; I, 32.0%.)

Palitantin monoxide. When palitantin is treated with a $CHCl_3$ solution of camphoric acid peracid at room temperature (3.8 g. in 100 ml. $CHCl_3$ solution as determined by thiosulphate titration—Milas & Cliff, 1933), oxidation takes place corresponding roughly with the oxidation of one double bond in 1 hr. and two double bonds in 20 hr.

Palitantin (0.25 g.) was treated with an excess (25 ml.) of camphoric acid peracid solution in $CHCl_3$ and held for 1 hr. at room temperature. The palitantin gradually dissolved and had completely dissolved in less than 1 hr. The mixture was then thoroughly extracted with aqueous $NaHCO_3$. On removal of the solvent from the $CHCl_3$ layer, a colourless solid remained which was recrystallized from ether (50 ml.). *Palitantin monoxide* was thus obtained as beautiful colourless clusters of needles (0.03 g.), m.p. 144°. It is fairly readily soluble in water. (Found: C, 62.7, 62.9; H, 8.3, 8.3. $C_{14}H_{22}O_5$ requires: C, 62.2; H, 8.2%.)

A solution of palitantin (1 g.) in 100 ml. of a $CHCl_3$ solution of camphoric acid peracid was held at room temperature for 18 hr. The oxygen absorbed corresponded almost exactly to 2 atoms/mol. of palitantin. The oxidation product, isolated as described in the previous paragraph, proved to be mainly an uncrystallizable oil containing 0.06 g. of palitantin monoxide, m.p. 137°. (Found: C, 61.9, 61.8; H, 8.2, 8.0%. $C_{14}H_{22}O_5$ requires: C, 62.2; H, 8.2%.)

Tetrahydropalitantin bis-p-bromobenzoate. A mixture of tetrahydropalitantin (0.25 g.), *p*-bromobenzoyl chloride (0.8 g.) and pyridine (5 ml.) was heated on a boiling-water bath for 10 min. Crystals formed on cooling. Water was added and decanted after a time. The residue was thoroughly extracted with aqueous $NaHCO_3$ and finally with water. The sticky residue was boiled with ethanol (30 ml.) and the residual crystals were separated and identified as bromobenzoic anhydride. By the cautious addition of water to the ethanolic mother liquors a sticky first crop of crystals was obtained which was rejected. The second crop was crystalline and, after recrystallization from water, *tetrahydropalitantin bis-p-bromobenzoate* was obtained as colourless clusters of flattened needles, m.p. 94.5°. (Found: C, 53.9, 53.9; H, 5.2, 5.3; Br, 25.4. $C_{28}H_{28}O_8Br_2$ requires: C, 53.8; H, 5.2; Br, 25.6%.)

Breakdown products and their derivatives

Hydrolysis of the dihydrazide $C_{13}H_{23}O_3N_4$ obtained by oxidation of tetrahydropalitantin with silver oxide

A solution of the dihydrazide (Birkinshaw & Raistrick, 1936, p. 807). (0.025 g., m.p. 190°) in 10N- H_2SO_4 (5 ml.) was boiled under reflux for 0.5 hr. After cooling, the hydrolysate was diluted with water (20 ml.) and thoroughly extracted with ether. On removal of the solvent and thorough drying *in vacuo*, a syrup (0.0185 g.) remained and showed no signs of crystallization. It was dissolved in ethanol (1 ml.) and titrated in the cold to phenolphthalein with 0.1N-NaOH; 0.81 ml. of 0.1N-NaOH were required, corresponding to an equivalent of 228. A mono-acid monolactone of the formula $C_{13}H_{22}O_4$, titrating as a monobasic acid, requires 242.

An excess (2.00 ml.) of 0.1N-NaOH was now added and the whole was heated on the water bath for 1 hr. After cooling, 1.27 ml. of 0.1N-HCl was required to neutralize to phenolphthalein. Hence the additional alkali used in the hydrolysis = 0.73 ml., giving a total amount of alkali used of 0.81 + 0.73 ml. = 1.54 ml. 0.1N. This corresponds to a total equivalent of 120. $C_{13}H_{22}O_4$, i.e. the mono-lactone of $C_{13}H_{24}O_5$, titrating as a dibasic acid requires an equivalent of 121.

Oxidative fission of palitantic acid. Formation of n-butyraldehyde

With $KMnO_4$. A solution of $KMnO_4$ (2 g.) in water (200 ml.) was added dropwise to a solution of palitantic acid (1 g.) in water (150 ml.) and N-NaOH (4 ml.). During the oxidation a brisk current of air was passed through the solution, and the issuing gas was scrubbed through bubblers containing Brady's reagent (2:4-dinitrophenylhydrazine in 2N-HCl). The resulting yellow precipitate was collected, dried and crystallized from methanol. Yellow needles, m.p. 123.5°, not depressed on admixture with authentic *n*-butyraldehyde 2:4-dinitrophenylhydrazone, m.p. 123°. (Found: C, 47.4, 47.4; H, 4.9, 4.8; N, 21.8, 22.0. Calc. for $C_{10}H_{12}O_4N_4$: C, 47.6; H, 4.8; N, 22.2%.) No other pure products could be isolated from the oxidation solution.

Ozonolysis. Ozonized O_3 was passed for about 7 hr. through a solution of palitantic acid (0.54 g.) in 0.1N-NaOH (20 ml.) and water (40 ml.). The mixture was now heated to about 60° and a current of air was bubbled through it and then through Brady's reagent. The resulting yellow precipitate proved to be *n*-butyraldehyde 2:4-dinitrophenylhydrazone. 0.42 g. of impure palitantic acid was recovered from the ozonized solution.

Oxidation of the lactonic acid $C_{13}H_{22}O_4$ from tetrahydropalitantin with nitric acid. Formation of (+)-n-heptylsuccinic acid

The syrupy lactonic acid from tetrahydropalitantin (1.1 g.; Birkinshaw & Raistrick, 1936, p. 807) was heated on the water bath with a mixture of conc. HNO_3 (13 ml.) and water (9 ml.). The reaction was vigorous at first, but gradually slackened and became negligible after 3 hr. The mixture was evaporated on the water bath in an open dish to a syrup. A smell of fatty acids, butyric, hexanoic, etc., was observed. On cooling, a mass of crystals mixed with oil was obtained, wt. 0.90 g. This mass was treated with a little water, well stirred and filtered. The filtrate, which consisted

of an aqueous solution with drops of oil suspended in it, contained much oxalic acid.

The solid (wt. 0.44 g.; m.p. 39–42°) was shown by titration to contain a little unchanged lactic acid. This was removed by repeated solution of the crystals in the titratable amount of *N*-NaOH followed by acidification with HCl. The final product, having a titration equivalent of 111, was recrystallized from ether (1 vol.) followed by the addition of light petroleum (b.p. 40–60°; 10 vol.). The oxidation product was thus obtained as colourless slender rods, m.p. 82–84°, not depressed on admixture with synthetic (±)-*n*-heptylsuccinic acid, m.p. 86° (see next section). (Found: C, 61.1, 61.1; H, 9.0, 9.3; equivalent by titration 108.7. Calc. for $C_{11}H_{20}O_4$: C, 61.1; H, 9.3%; equivalent, titrating as a dicarboxylic acid, 108.1.) $[\alpha]_{D}^{20} = +33^\circ$ (c, 0.97 in ethanol).

Optically active (+)-n-heptylsuccinic acid dihydrazide. The oxidation acid (0.2 g.) was esterified with diazomethane and treated with a solution of hydrazine hydrate (50%; 0.5 ml.) in ethanol (1 ml.). The crystals which appeared overnight were recrystallized from ethanol-ether and were obtained as colourless needles, m.p. 166°. (Found by I.C.I. Butterwick Research Laboratories: C, 53.8, 54.0; H, 9.9, 9.8; N, 22.3, 22.5. $C_{11}H_{24}O_2N_4$ requires C, 54.1; H, 9.9; N, 22.9%.)

Synthesis of (±)-*n*-heptylsuccinic acid

Ethyl 2-bromononanoate (α -bromopelargonate) was condensed with ethyl sodiomalonate, and the resulting triester was hydrolysed and heated to eliminate one carboxyl group.

Preparation of ethyl 2-bromononanoate (Auwers & Bernhardt, 1891). Bromine (10 ml.) was added drop by drop to a mixture of nonanoic acid (15.8 g.) and red P (1.04 g.). The mixture was gradually warmed to 90–100° and more Br (2 ml.) was added. When the evolution of HBr had practically ceased (4–5 hr.) the mixture was cooled and ethanol (30 ml.) was added with cooling. Next day the ethyl α -bromopelargonate was extracted with ether and the ethereal solution was washed with water and aqueous Na_2CO_3 , dried over Na_2SO_4 and distilled *in vacuo*. The ester (22.45 g.) was collected at 135–142°/15 mm.

Condensation of ethyl 2-bromononanoate with malonic ester and hydrolysis of the resulting triester. Na (1.96 g.) was dissolved in ethanol (27 ml.) and malonic ester (13.6 g. = 11.8 ml.) was added. The mixture was warmed slightly and ethyl 2-bromononanoate (22.4 g.) was added, and the whole was refluxed for 2 hr. The resulting ester was extracted in the usual manner and distilled *in vacuo*. Yield 21.7 g.; b.p. 153°/1 mm.

The ester (2 g.) was heated with KOH (5 g.) and water (3 ml.) in a nickel crucible with constant stirring, the temperature of the metal bath being raised to 300° as rapidly as frothing would permit. The melt was cooled, dissolved in water and acidified with HCl. Colourless plates (1.27 g.) separated, m.p. 126–128°.

Decarboxylation of the tricarboxylic acid. The above acid, m.p. 126–128°, was heated in an oil bath when decarboxylation occurred at the melting point (bath temp. 135–140°). After about 10 min. effervescence had practically ceased. The product which crystallized on cooling was neutralized with aqueous *N*-NaOH, filtered and acidified with HCl. The resulting emulsion soon crystallized. The crystals were collected, dried and recrystallized from ether-light petroleum.

(±)-*n*-Heptylsuccinic acid was thus obtained as colourless irregular prisms, m.p. 86°; equivalent by titration 110. (Found by I.C.I. Butterwick Research Laboratories: C, 61.0, 61.0; H, 9.0, 9.2. $C_{11}H_{20}O_4$ requires C, 61.1; H, 9.3%; equivalent 113.)

(±)-*n*-Heptylsuccinic acid dihydrazide. This dihydrazide was prepared in the same way as was the dihydrazide of the oxidation product (+)-*n*-heptylsuccinic acid (see above). It formed colourless needles, m.p. 156°. Although there was a difference of 10° in melting point between the synthetic (±) and the natural dihydrazides, there was no depression in melting point on mixing them.

Reactions with periodate

Action of periodate on tetrahydropalitanin

Oxygen utilization. Tetrahydropalitanin (0.0403 g.) dissolved in water (100 ml.) by warming and subsequent cooling was treated with KIO_4 (50 ml. of 0.0125 *M*) and left overnight. Excess H_2SO_4 and KI were then added and the I_2 liberated was titrated with 0.05 *N*- $Na_2S_2O_3$. The I_2 deficit as compared with a blank experiment was 6.24 ml. of 0.1 *N*, equivalent to a disappearance of four atoms I or utilization of two atoms O/mol. of tetrahydropalitanin.

Acidic groups formed. The experiment was repeated with 0.1616 g. of tetrahydropalitanin using the theoretical amount (100 ml.) of KIO_4 (0.0125 *M*), in order to determine the acid and carbonyl groups present after the reaction. After standing overnight, the solution required 11.54 ml. of 0.1 *N*-NaOH for neutralization to phenolphthalein. The theoretical figure for 2 equivalents of acid produced in the reaction would be 12.50 ml. The actual figure obtained was always somewhat lower, doubtless owing to lactone formation.

Aldehyde titration

By Doeuvre's method (Doeuvre, 1927). To the neutralized solution was added mercury potassium iodide (3 g.) in a little water with just sufficient KI to obtain complete solution, followed by NaOH (40 ml. of 0.1 *N*). A yellow precipitate was formed in the solution. After 3 hr., titration with 0.1 *N*-acetic acid containing equimolar sodium acetate showed the disappearance of 16.05 ml. of alkali. The aldehyde present is equivalent to 5.35 ml. ($= \frac{1}{3}$ of 16.05), whereas the theoretical value for 1 CHO = 6.25 ml.

By bisulphite titration (Malaprade (1934) method). The periodate oxidation was repeated (0.1616 g. of tetrahydropalitanin; 100 ml. of 0.0125 *M*- KIO_4). 11.20 ml. of 0.1 *N*-NaOH were required for neutralization of the acid produced in the reaction. Bisulphite (30 ml. about 0.2 *M*) was then added, the solution was left for 1 hr. with exclusion of air, and was then titrated. It required 15.90 ml. of 0.05 *M*-iodate. The bisulphite taken was equivalent (by titration) to 45.04 ml. of 0.05 *M*-iodate, the difference being 29.14 ml. Of this 25.00 ml. would be derived from the periodate. The iodate corresponding with aldehyde is, therefore, 4.14 ml. whereas 1 CHO group would require 4.17 ml.

By hydroxylamine titration (Schultes, 1934). The same method of oxidation was followed and the same quantities used, but after removal of the iodate with H_2SO_4 and KI and titration of the liberated I_2 with thiosulphate, the solution was neutralized to bromophenol blue, and a solution of about 1 g. of NH_4OH , HCl neutralized to the same end point was added. The mixture immediately became acid and was

titrated from time to time with 0.1N-NaOH until permanent neutrality (to the same end point) was reached. The alkali used was 6.7 ml., whereas 1 CHO group requires 6.25 ml.

There is ample evidence to show the production (or persistence) of 1 CHO group on periodate oxidation.

Production of lactone group. The production of a lactone group is shown more clearly in the following experiment. Tetrahydropalitantin (0.25 g.) in 25 ml. of methanol was treated with KIO_4 (0.5 g.) in water (125 ml.). A turbidity appeared in 5–10 min. which gradually increased. After standing overnight the mixture was titrated to phenolphthalein. It used 16.8 ml. of 0.1N-NaOH to the first pink flush, but required 5–6 hr. to reach a permanent end point, when 20.5 ml. of NaOH had been utilized, the theoretical figure for 2 equiv. of acid produced being 19.4 ml. After standing overnight a further 0.30 ml. of 0.1N alkali was required.

Characterization of the periodate oxidation product of tetrahydropalitantin. The product obtained by the oxidation of tetrahydropalitantin (0.25 g.) with KIO_4 (0.5 g.) was extracted with ether, suspended in water (100 ml.) and treated with Doeuve's reagent (0.45 g. of HgI_2 , 2 g. of KI in a little water). Then N-NaOH (10 ml.) was introduced, the mixture was well shaken and left overnight. The precipitated Hg was filtered off and the solution titrated. It required 6.40 ml. of N-HCl; the acid produced in the reaction is therefore 3.60 ml. (1 CHO group requires 3.88 ml.). The solution was now acidified with HCl and extracted with ether. The ether extract was esterified by means of diazomethane. The resulting oil (which contained a little Hg salt) was treated with ethanol (2 ml.) and hydrazine hydrate (50%, 1 ml.). The solid mass of crystals obtained (0.17 g.) was drained at the pump, and recrystallized from ethanol giving 0.12 g. of pure product of m.p. 190°. This gave no depression when mixed with the product $\text{C}_{13}\text{H}_{28}\text{O}_3\text{N}_4$ obtained previously by the oxidation of tetrahydropalitantin with Ag_2O (Birkinshaw & Raistrick, 1936, p. 807).

In a larger scale experiment tetrahydropalitantin (1.613 g.) was oxidized by KIO_4 (2.875 g.) and the aldehydic product further oxidized with Doeuve's reagent as above. In this case the mercury precipitated was weighed and acid produced in the Doeuve reaction was determined (Hg 1.23 g. 1 CHO requires 1.25 g.; acid formed 17.9 ml. 1 CHO requires 18.75 ml.).

The solution was acidified to Congo red with H_2SO_4 and extracted with CHCl_3 (4×2 l.). After removal of solvent *in vacuo* the residue (1.22 g.) was taken up in ether, filtered from a small amount of HgI_2 , taken up by the CHCl_3 and esterified by means of diazomethane. The product, distilled *in vacuo* afforded 0.84 g. of distillate b.p. 175°/3 mm. with methoxyl content 12.6%. $\text{C}_{14}\text{H}_{24}\text{O}_4$ requires (for 1 OMe) 12.1%. This represents the monomethyl ester of the lactone acid.

The distillate (0.79 g.) was treated with phenylhydrazine (0.5 g.) in ethanol (2 ml.) and heated under reflux for 2 hr. The product was treated with ether and the solvent allowed to evaporate slowly. A small amount of crystals was obtained, m.p. 192°, raised on recrystallization to 206°. (Found: C, 67.8; H, 8.2; N, 12.8. $\text{C}_{23}\text{H}_{36}\text{O}_3\text{N}_4$ requires C, 68.1; H, 8.2; N, 12.7%. The product is evidently the *bisphenylhydrazide* of the lactonic acid $\text{C}_{13}\text{H}_{22}\text{O}_4$.)

Action of periodate on palitantin

Palitantin (0.0594 g.) in ethanol (25 ml.) was treated with KIO_4 (0.0125M; 100 ml.) and H_2SO_4 (2N; 10 ml.). After 20 hr. at room temperature excess KI was added and the I_2 was titrated with 0.1N- $\text{Na}_2\text{S}_2\text{O}_3$. The I_2 deficit as compared with a blank was 13.03 ml. 0.1N corresponding with 5.74 atoms I/mol. of palitantin. Part of the I_2 uptake is due to I_2 substitution, which is a characteristic property of palitantin and presumably of the fission product.

When the theoretical amount of KIO_4 for an uptake of 4 I atoms (=20) was used and there was no subsequent liberation of free I_2 the results were similar to those obtained with tetrahydropalitantin.

Evidence of lactone formation. Palitantin (1 g.) in methanol (100 ml.) treated with KIO_4 (2 g.) in water (600 ml.) remained clear. After 24 hr. it required 6.4 ml. of N-NaOH to the first pink flush of phenolphthalein gradually increasing to 7.95 ml. over 3 hr. The theoretical value for 2 equivalents of acid produced is 7.87 ml. This is again evidence for one product being a lactone.

Aldehyde production. The reaction mixture was acidified with 10 ml. of 2N- H_2SO_4 and extracted with ether. The oily residue obtained on evaporation of the ether was suspended in water (about 200 ml.) and neutralized to bromophenol blue. After addition of a solution of $\text{NH}_4\text{OH} \cdot \text{HCl}$ (2 g.) at the same pH the solution was titrated at intervals until a permanent end point was reached, matching to the same arbitrary standard. The NaOH required was 3.4 ml., the theoretical amount for 1 aldehyde group being 3.88 ml. Practically all the emulsion dissolved leaving only a trace of turbidity.

Oxidation of aldehyde group and characterization of product. The product from the KIO_4 (2 g.) oxidation of palitantin (1 g.) was extracted with ether after acidifying. The residual syrup after removal of ether was oxidized with Doeuve's reagent. Barium acetate was added and the oxidation product was precipitated as the barium salt by addition of 4 vol. of ethanol. The Ba precipitate was collected, suspended in water, acidified with HCl and the acid product extracted with ether. The product was then esterified by means of diazomethane and treated with ethanol (4 ml.) and hydrazine hydrate (50%; 2 ml.). The crystals obtained (0.47 g.; m.p. 202–204°) showed no depression when mixed with the dihydrazide $\text{C}_{13}\text{H}_{24}\text{O}_3\text{N}_4$ obtained by oxidation of palitantin with Ag_2O as previously described (Birkinshaw & Raistrick, 1936).

Action of periodate on tetrahydropalitantic acid

Utilization of oxygen. Tetrahydropalitantic acid (0.0857 g.) suspended in water (25 ml.) was neutralized to phenolphthalein to bring it into solution and treated with KIO_4 (0.0125M; 50 ml.) and left overnight. Excess of H_2SO_4 and KI were then added and the liberated I_2 was titrated with $\text{Na}_2\text{S}_2\text{O}_3$. The deficiency of I_2 was 6.44 ml. of 0.1N as compared with a blank, corresponding with a utilization of 2.06 atoms of I ($\equiv 1.03$ atoms of O).

Determination of acid and carbonyl groups. Tetrahydropalitantic acid (0.1713 g.) was dissolved in the theoretical amount of NaOH (0.1N; 6.25 ml.) and KIO_4 (0.0125M; 50 ml.; i.e. the theoretical amount) was added. After 2 days it required 0.11 ml. of 0.1N-NaOH to neutralize to phenolphthalein, i.e. no acid is formed in the reaction. To the titrated solution were added H_2SO_4 (0.1N; 50 ml.) and

excess KI. Titration with $\text{Na}_2\text{S}_2\text{O}_3$ showed a deficiency of 12.48 ml. of 0.1N- I_2 corresponding with 2 atoms of I (theoretical 12.50 ml.). The solution was then treated with bromophenol blue and brought to an arbitrary end point (faint purple colour) with NaOH. NH_4OH , HCl (1 g. in 10 ml. of water) neutralized to the same end point was added. After 2 hr. the alkali required to establish the same end point was 11.5 ml. of 0.1N-NaOH. The theoretical value for two aldehyde groups is 12.5 ml. Thus two carbonyl groups are produced in the reaction, provided no such groups titratable with NH_4OH are present in the original tetrahydropalitanic acid. The absence of such groups was shown by the addition of the NH_4OH reagent to another sample of the acid. No acidity was produced.

Isolation of frequentin

The crude crystalline product (11.75 g.) obtained by CHCl_3 extraction of the culture solution from two batches of *P. palitans* type strain (Birkinshaw & Raistrick, 1936) was dissolved in CHCl_3 (500 ml.) and 100 ml. of water were added. N-NaOH was added dropwise with vigorous shaking until the aqueous layer was just alkaline to phenolphthalein. This required 11.5 ml. of N-NaOH. The aqueous layer was separated and acidified with N-HCl (12 ml.). The oil precipitated solidified on chilling and was crude frequentin, wt. 2.52 g. A small amount was recovered from the mother liquor.

Frequentin was difficult to recrystallize as it decomposed on heating for any length of time. It was purified for analysis by recrystallizing in small amounts from boiling water, with addition of decolorizing charcoal. Frequentin crystallized in colourless needles, m.p. 134.5° (decomp.), $[\alpha]_{\text{D}}^{20} +82^\circ$ in CHCl_3 . On microtitration 10.260 mg. required 2.80 ml. N/70-NaOH, equiv. 257. (Found: C, 66.0, 66.0, 66.5, 66.4; H, 7.7, 7.8, 8.2, 8.2. Calc. for $\text{C}_{14}\text{H}_{20}\text{O}_4$: C, 66.6; H, 8.0%, equiv. 252.) Frequentin gives a reddish

purple colour with FeCl_3 in a aqueous or alcoholic solution and yields a precipitate with Brady's reagent.

A comparison of our product obtained from *P. palitans* Westling with frequentin obtained from *P. frequentans* (Curtis *et al.* 1951), kindly carried out by Mr P. J. Curtis, indicated that the two products were identical since there was no depression in melting point on mixing.

SUMMARY

1. A number of derivatives and breakdown products of palitantin have been prepared and are described.

2. Palitantin, contrary to the previous statement (Birkinshaw & Raistrick, 1936), has a slight but definite optical activity.

3. The oxidation of palitantin and its derivatives with periodate has been studied in a quantitative manner.

4. Tetrahydropalitanin probably consists of a 6-carbon ring with a *n*-heptyl and a formyl group as substituents and with hydroxyl groups situated α and β to the formyl group. In palitantin one double bond is in the C_7 chain in the γ position to the terminal methyl group. The function of the fourth oxygen atom and position of the second double bond (if present) are still obscure.

5. The acidic substance which accompanies palitantin as a metabolic product of *Penicillium palitans* is identical with frequentin isolated from *Penicillium frequentans* by Curtis *et al.* (1951).

I have to thank the Butterwick Research Laboratories of I.C.I. Ltd. for the elementary analyses of two products. Other micro-analyses are by Weiler and Strauss, Oxford.

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A Structural Relationship between Frequentin and Palitantin

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(Received 19 October 1951)

Birkinshaw & Raistrick (1936), in describing the isolation of palitantin ($\text{C}_{14}\text{H}_{22}\text{O}_4$) from *Penicillium palitans*, mention the occurrence of a dextrorotatory acid in concentrates from one strain. Curtis, Hemming & Smith (1951) isolated a substance named frequentin ($\text{C}_{14}\text{H}_{20}\text{O}_4$) together with palitantin from certain atypical strains of *Penicillium frequentans*. Subsequently it was pointed out to us

by Dr J. H. Birkinshaw (cf. Birkinshaw, 1952, preceding paper) that the dextro-rotatory acid was very probably identical with frequentin, and we have established this by a mixed melting point determination and by comparing the infrared spectra of the two substances.

In common with palitantin, frequentin has an aldehyde group, an ethylenic double bond or bonds