CXXXI. STUDIES IN THE BIOCHEMISTRY OF MICRO-ORGANISMS

LIV. THE MOLECULAR CONSTITUTION OF TERREIN, A METABOLIC PRODUCT OF *ASPERGILLUS* *TERREUS* THOM

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RAISTRICK & SMITH [1935] have shown that two strains of the group species *Aspergillus terreus* Thom, when grown at 24° on Czapek-Dox solution containing glucose as the sole source of carbon, give rise to a hitherto undescribed mould metabolic product—terrein. It is the purpose of the present communication to describe the investigation of the molecular constitution of this substance.

Terrein, $C_8H_{10}O_3$, is a colourless, crystalline, dextrorotatory, neutral substance having powerful reducing properties. The condition of the 3 oxygen atoms present in the molecule of terrein is indicated as follows. (a) One is present as a hydroxyl group since terrein readily gives a mono-*p*-bromobenzoate. (b) One is present as a ketone group since terrein readily gives a mono-2:4-dinitrophenylhydrazone, and the keto-group can be quantitatively titrated with hydroxylamine hydrochloride by Schultes's method [1934]. The presence of an aldehyde group is excluded since terrein does not give a positive Schiff or Angeli-Rimini reaction. (c) The CO— and CHO— groups are probably adjacent in a —CO.CHO— grouping since terrein instantly reduces Fehling's solution in the cold and slowly gives a bisdinitrophenylhydrazone on treatment with 2:4-dinitrophenylhydrazine. In this reaction 2 atoms of hydrogen are lost suggesting the conversion of a —CO.CHO— into a —CO.CO— grouping, as is the case with acetoin [Clutterbuck *et al.* 1935]. (d) In a Zerewitinoff estimation terrein gave in pyridine 1.39 mol. CH_4 at 19° and 2.06 mol. at 28°, suggesting the presence of one OH and one enolizing CO group. (e) No direct evidence has been obtained of the condition of the third oxygen atom.

These considerations, together with the following groups of facts A, B, C, D and E, have led to the present conception of the molecular constitution of terrein.

A. Terrein, on catalytic reduction with palladium-charcoal-hydrogen very quickly absorbs 2 mol. of hydrogen to give a crystalline product, tetrahydroterrein, $C_8H_{14}O_3$ (structure III), which is strongly laevorotatory and instantly reduces Fehling's solution in the cold. It gives a monodinitrobenzoate and a bisdinitrophenylhydrazone. The —CO.CHO— grouping in terrein is therefore probably intact in tetrahydroterrein. Tetrahydroterrein, on oxidation with HIO_4 by the Malaprade [1928] method, absorbs 2 atoms of oxygen and yields 1 mol. of formic acid and 1 mol. of a monobasic acid $C_7H_{12}O_3$ according to the equation

The monobasic acid $C_7H_{12}O_3$ gives a monodinitrophenylhydrazone soluble in $NaHCO_3$ and an ethyl ester monodinitrophenylhydrazone insoluble in $NaHCO_3$. It is almost certainly, therefore, an aldehydo-acid and on oxidation with alkaline iodine it gives rise to *d-n*-propylsuccinic acid $C_7H_{12}O_4$ (VI). The acid $C_7H_{12}O_3$ must therefore have either structure I or II, and tetrahydroterrein, using the analogies worked out in an earlier paper [Clutterbuck & Reuter, 1935], must have either structure V (arising from I) or III (arising from II).

B. That tetrahydroterrein has structure III and not structure V follows from a study of its reactions. Its most characteristic property is the ease with which it loses a molecule of water. Thus on boiling it with *N* H_2SO_4 there is formed an α -diketone $C_8H_{12}O_2$ which has been shown by synthesis to be 4-propyl-2-ketocyclopentanone (XI). This compound gives a bisdinitrophenylhydrazone and a monodinitrobenzoate identical with the products arising directly from tetrahydroterrein on treatment with the same reagents. It is, however, optically inactive, titrates as a monobasic acid, gives an intense violet $FeCl_3$ colour and does not reduce Fehling's solution except on heating, whereas tetrahydroterrein is a laevorotatory, neutral substance, giving no colour with $FeCl_3$, but reducing Fehling's solution instantly in the cold. On catalytic reduction with $Pd-C-H_2$, the diketone $C_8H_{12}O_2$ (XI) very quickly absorbs 2 atoms of hydrogen (as does diacetyl) to give an oil, $C_8H_{14}O_2$ (XIII), which is no longer acidic, no longer gives a $FeCl_3$ colour, but reduces Fehling's solution instantly in the cold. With 2:4-dinitrophenylhydrazine it gives at first a monodinitrophenylhydrazone, followed slowly by the production of a bis-derivative (in which 2 atoms of hydrogen are lost) which is identical with that from the unreduced diketone $C_8H_{12}O_2$ (XI). These facts are readily explained by supposing that the α -diketone grouping, in its enolized form (XII), is converted by reduction into the strongly reducing α -hydroxyketone grouping

Accepting structure (XI) as shown by synthesis for the diketone $C_8H_{12}O_2$, then tetrahydroterrein must be represented by structure (III). This conclusion is supported by the fact that it offers a logical explanation for the observation that laevorotatory tetrahydroterrein, on loss of H_2O by heating with dilute H_2SO_4 , gives an optically inactive diketone (XI), since structure (III), on loss of H_2O , leads to a molecule which is symmetrical about the 4-C atom to which the propyl group is attached, whereas structure (V) leads to an unsymmetrical molecule. Hence tetrahydroterrein must be formulated as 4-propyl-2:3-dihydroxycyclopentanone (III), the acidic diketone $C_8H_{12}O_2$ as 4-propyl-2-ketocyclopentanone (XI) in equilibrium with its enolic form (XII), which on reduction gives the hydroxyketone $C_8H_{14}O_2$, i.e. 4-propyl-2-hydroxycyclopentanone (XIII).

The fact that tetrahydroterrein (III) and the diketone (XI) give the same monodinitrobenzoate and bisdinitrophenylhydrazone is then readily explained by assuming that during the formation of these derivatives the elements of H_2O are removed from (III) in the same way as occurs on warming (III) with dilute H_2SO_4 . The behaviour of tetrahydroterrein on boiling with *N* H_2SO_4 closely resembles that of 3-methyl-2:3-dihydroxycyclohexanone [Harries, 1902] which similarly loses H_2O under the same conditions to give 3-methyl-2-ketocyclohexanone. This compound, like the diketone $C_8H_{12}O_2$ (XI), behaves on titration as an acidic diketone and gives an intense violet $FeCl_3$ colour.

The oxidation of tetrahydroterrein (III) with HIO_4 , to give an aldehydo-acid $C_7H_{12}O_3$ (II), which on treatment with alkaline iodine gives *d-n*-propylsuccinic

acid (VI), is then explained by postulating the intermediate formation of the dialdehyde (IV), leading to the formation of formic acid and the aldehydo-acid (II), which with alkaline iodine gives (VI). Analogies for the reaction with HIO_4 have already been published [Clutterbuck & Reuter, 1935], and the oxidative opening of such reduced ring systems by HIO_4 has recently been used by Fischer & Dangschat [1934] in the conversion of quinic acid into citric acid.

C. Tetrahydroterrein (III) on distillation at atmospheric pressure loses 1 H_2O to give rise to at least two products, one of which, formed only to a small extent, is 4-propyl-2-ketocyclopentanone (XI), this reaction being identical with that on boiling with $N \text{H}_2\text{SO}_4$. The second and chief reaction results in the formation of 4-propyl-3-ketocyclopentanone (VII). As a result of the presence of this compound (VII) distilled tetrahydroterrein is optically active. Distilled tetrahydroterrein with Pd-C-H_2 readily absorbs 2 atoms of hydrogen giving an oil which is no longer acidic and does not give a FeCl_3 colour. This oil, and also the oil obtained by protracted reduction of tetrahydroterrein itself, deposits on standing crystals of 4-propyl-3-hydroxycyclopentanone $\text{C}_8\text{H}_{14}\text{O}_2$ (X), which on oxidation with KMnO_4 gives *d-n*-propylsuccinic acid (VI), and with HNO_3 a lactonic acid $\text{C}_8\text{H}_{12}\text{O}_4$, which is probably the γ -lactone of α -hydroxy- β -*n*-propylglutaric acid (IX), since on oxidation with Kiliani's mixture it gives *d-n*-propylsuccinic acid (VI).

D. Since tetrahydroterrein (III) arises from terrein by the addition of 4 atoms of hydrogen, it now remains to determine the positions of attachment of these 4 hydrogen atoms. The positions of two of them may be inferred from the fact that on treatment with ozone terrein forms an ozonide which on warming is decomposed with the liberation of acetaldehyde. It appears certain, therefore, that the propyl side-chain of tetrahydroterrein is present in terrein as a propenyl group $\text{CH}_3\cdot\text{CH}=\text{CH}-$, and that the constitution of the intermediate and at present unisolated dihydroterrein is represented by structure (VIII).

E. The removal of 2 hydrogen atoms from structure (VIII) to give a formula explaining all the facts known about terrein presents some difficulties. Eight possible ways of effecting the removal are shown in the chart. Thus 2 hydrogen atoms may be removed in such a way that three substances each having two double bonds are formed, i.e. (XIV), (XV), (XVI); or a CO group may arise from a CHOH group giving the two ketonic structures (XVII) and (XVIII); or the hydrogens may be removed in such a way that oxygen-bridged structures would arise, i.e. (XIX), (XXIII), (XXVII).

In their reactions with HIO_4 tetrahydroterrein and terrein each absorb 2 atoms of oxygen and give formic acid, but whereas tetrahydroterrein gives the optically active half aldehyde of *d-n*-propylsuccinic acid (II), terrein gives the optically inactive half aldehyde of propenylfumaric acid which is represented in the lactonized form in (XXV) and which on reduction with Pd-C-H_2 rapidly absorbs 2H_2 and slowly a third mol. of hydrogen with loss of an oxygen atom and gives β -propylbutyrolactone (XXVI). This substance was synthesized for comparison.

The production of propenylfumaric acid half aldehyde (XXV) from terrein by HIO_4 , with the absorption of 2 atoms of O, rules out formulae (XIV), (XVI), (XVII) and (XVIII). Thus (XIV) would give propadienesuccinic acid half aldehyde and $\text{H}\cdot\text{COOH}$; (XVI) and (XVII) would each give propenylsuccinic acid and $\text{H}\cdot\text{COOH}$ and (XVIII) would give propenylsuccinic acid half aldehyde and CO_2 . Of the oxygen-bridged structures, since a $\text{CO}\cdot\text{CHOH}$ group is regarded as an essential part of the structure, (XIX) and (XXVII) are improbable and moreover the oxygen bridge would require to open before a reaction could occur at all.

We are left therefore with formulae (XV) and (XXIII) as the most probable structures for terrein.

If formula (XV) is accepted, it must be assumed that on reduction of terrein to tetrahydroterrein the optically active CHOH groups so direct reduction that a new asymmetric centre arises at the carbon atom in position 4, the product then giving optically active propylsuccinic acid on oxidation. It must be assumed also that, since tetrahydroterrein so readily loses water to give the acidic diketone (XI) whereas terrein does not, the presence of the double bond in the ring stabilizes in some way the CO.CHOH.CHOH group so that it no longer loses water. This appears somewhat unlikely. It would also be necessary to explain the fact that terrein gives only a mono-*p*-bromobenzoate. Moreover, formula (XV), owing to the conjugation of the double bonds, might be expected to give a compound with maleic anhydride, but no such compound is formed even when terrein is melted with maleic anhydride.

If formula (XXIII) is accepted we must assume the following changes in order to explain the production by HIO_4 of the half aldehyde of propenylfumaric acid. First HIO_4 attacks the CO.CHOH group in the usual way, opening the cyclopentane ring and giving the aldehydo-acid (XXIV). Next the oxygen bridge opens creating a new CHOH.CHO group (XXI), which in the usual way loses H.COOH giving the aldehydo-acid (XXII) which lactonizes. Finally the tertiary hydrogen atom at carbon 4 together with the hydroxyl group at carbon 5 are lost as H_2O giving (XXV). Accepting this series of reactions as likely, the most probable structure of terrein becomes (XXIII).

EXPERIMENTAL

General properties of terrein

The terrein used throughout this work was prepared as described by Raistrick & Smith [1935]. Terrein, $\text{C}_8\text{H}_{10}\text{O}_3$, M.P. 127° , $[\alpha]_{5461}^{20} + 185^\circ$ (1% solution in water) is a colourless neutral substance, having powerful reducing properties. Thus it decolorizes alkaline phenolphthalein, reduces Fehling's solution and ammoniacal AgNO_3 instantly in the cold and cupric acetate on boiling. It is not, however, as powerfully reducing, e.g. as ascorbic acid, since it reacts only very slowly with 2:6-dichlorophenolindophenol and cannot be titrated either with this reagent or with acid iodine. An aqueous solution of terrein on adding *N*/10 NaOH (but not 2*N* Na_2CO_3) gives a bright yellow colour which is discharged on immediate acidification. On standing in alkaline solution for a longer time, a brown colour is obtained which is no longer discharged with acid. Terrein gives no colour with NaNO_2 , does not give a positive Schiff test and any colour developed on using the Angeli-Rimini technique is masked by the brown-yellow colour obtained with terrein in alkaline solution. It does not lose CO_2 on boiling for 5 hr. with 2*N* H_2SO_4 and has not therefore a tetrionic acid structure. Terrein on melting with maleic anhydride using the method of Morrell & Samuels [1932], did not form a condensation product and was recovered unchanged.

Terrein reacts in the cold with phenylhydrazine in acetic acid. On dilution with water an oil separates which rapidly solidifies, but the product could not be recrystallized. No immediate reaction takes place when terrein is treated with ethereal diazomethane, but on standing a slow reaction occurs with the formation of a black tar. Terrein, both in glacial acetic acid and chloroform solution absorbs bromine, but crystalline bromo-derivatives were not obtained.

In a Zerewitinoff estimation (Roth) it gave in anisole at 28° 1.60, 1.67 mol. CH_4 ; at 95° , 1.85, 1.84 mol.; in pyridine at 19° , 1.39 and at 28° , 2.06 mol. These

results are best explained in terms of the presence of 1 OH and 1 enolizing CO group.

Titration of terrein with hydroxylamine hydrochloride. The method followed was that of Schultes [1934]. Terrein (0.1052 g.) was added to a 20% aqueous solution (5 ml.) of hydroxylamine hydrochloride. The solution was titrated from time to time using bromophenol blue as indicator and the same amount of reagent was titrated alongside as a blank. After 6 hr. 6.6 ml. *N*/10 alkali were required (1 CO group requires 6.83 ml.). 0.1290 g. terrein after 24 hr. required 8.25 ml. *N*/10 NaOH (1 CO group requires 8.38 ml.) thus indicating that no further reaction occurred on standing. One CO group is therefore free to form an oxime.

*Terrein mono-*p*-bromobenzoate.* Terrein (0.25 g.), pyridine (5 ml.) and *p*-bromobenzoyl chloride (0.80 g.) were heated for 20 min. on a boiling water-bath and kept for 24 hr. at room temperature. The solution darkened on standing. The pyridine was evaporated *in vacuo* and the residue treated with NaHCO₃ solution when it gradually solidified. The washed and dried product weighed 0.49 g. (calc. for monobenzoate, 0.55 g.). The crude benzoate was ground with ether in which the pigmented material remained undissolved. The benzoate separated from ether in colourless needles, m.p. 145–146°. (Found (Schoeller): C, 53.15; H, 3.78; Br, 23.51%. C₁₅H₁₃O₄Br requires C, 53.42; H, 3.89; Br, 23.71%.)

Terrein mono- and di-2:4-dinitrophenylhydrazones. On adding an excess of Brady's reagent (2:4-dinitrophenylhydrazine in 2*N* HCl) to an aqueous solution of terrein (1 g.), a bright red precipitate is quickly formed, wt. after 1 day 2.30 g., after 1 week 2.90 g., after 5 weeks 3.62 g. (theory for mono-derivative 2.16 g., for di-, 3.34 g.).

Mono-2:4-dinitrophenylhydrazone. The precipitate obtained after 1 day, consisting chiefly of the mono-derivative, was filtered, washed and dried. The crude material was crystallized from alcohol by cooling to –20°, and after purification by this method it readily crystallized from ethyl acetate or methyl alcohol in dark red plates, m.p. 211°. It can also be readily prepared by using Brady's more recent technique [1931]. Thus on mixing terrein (0.5 g.) in alcohol (4 ml.) with a solution containing 2:4-dinitrophenylhydrazine (1 g.), concentrated H₂SO₄ (2 ml.) and alcohol (15 ml.) a crystalline precipitate of the mono-derivative separated at once. It gives with alcoholic NaOH the red colour characteristic of a mono-derivative (Neuberg's reaction). (Found (Schoeller) after drying *in vacuo* at 110°: C, 50.32; H, 4.42; N, 16.86%. C₁₄H₁₄O₆N₄ requires C, 50.29; H, 4.22; N, 16.77%.)

Bis-2:4-dinitrophenylhydrazone. The precipitate obtained after 5 weeks consists of a complex mixture of the bis-compound and a black tar. It was crystallized first from dioxan-alcohol-water, then from pyridine-acetic acid-water and finally from dioxan-acetic acid in dark reddish brown plates, m.p. above 360°. It gives the blue-violet Neuberg's reaction characteristic of a bis-derivative. (Found (Schoeller): N, 20.84%. C₂₀H₁₆O₈N₈, H₂O requires N, 21.13%.) It is probably the monohydrate of the bisdinitrophenylhydrazone of terrein after loss of H₂ by the conversion of a —CHOH— group into —CO— before condensation.

Catalytic reduction of terrein

Tetrahydroterrein (4-propyl-2:3-dihydroxycyclopentanone, III). When hydrogenated in aqueous solution using a Pd-norite catalyst, terrein rapidly absorbed 2H₂ (in 5 min.) and then gradually absorbed a third mol. in about 3 hr., the reaction then appearing complete. Terrein (4 g.) in water (100 ml.) was reduced in presence of a catalyst prepared from PdCl₂ (0.2 g.) and charcoal (1 g.). As soon

as 2H_2 had been absorbed the reaction was stopped, the hydrogen replaced by CO_2 , the catalyst filtered off and the aqueous solution continuously extracted with ether for 3 hr. The ethereal extract was dried over anhydrous MgSO_4 and concentrated to 25 ml. On cooling to -20° and scratching with a glass rod, or on nucleating, tetrahydroterrein separated in glistening colourless plates, wt. 2.5 g. From the mother-liquor, further small crops of crystals were obtained, but these were largely contaminated with an oil which drained on porous pot. The oil was then recovered by extraction of the pot with ether but was never obtained crystalline. The crystals were practically pure and were recrystallized from ether-light petroleum (B.P. $40-50^\circ$), M.P. 84° , unchanged after resetting. $[\alpha]_{\text{D}}^{18} -280^\circ$ ($c=0.379$ in water), not materially altered on standing for 24 hr. (Found (Schoeller): C, 60.76, 60.84; H, 8.75, 8.78%. $\text{C}_8\text{H}_{14}\text{O}_3$ requires C, 60.72; H, 8.92%.)

In a Zerewitinoff estimation (Roth) it gave 2.36 mol. of CH_4 in anisole and 2.40 mol. in pyridine in each case at 18° .

Tetrahydroterrein is very soluble in water and in most organic solvents, but is insoluble in light petroleum. It reduces Fehling's solution instantly in the cold, gives a yellow colour in alkaline solution and a very faint yellow FeCl_3 colour. After keeping for several months, it slowly loses H_2O , giving a yellow oil which gives an intense red-violet FeCl_3 colour. This yellow oil may be readily prepared by dehydration of tetrahydroterrein with H_2SO_4 .

Reaction between tetrahydroterrein and 2:4-dinitrophenylhydrazine. Tetrahydroterrein (0.038 g.) in water (5 ml.) was treated with an excess of Brady's reagent. A precipitate was formed which, on standing 48 hr., weighed 0.130 g. (theory, assuming the reaction $\text{C}_8\text{H}_{14}\text{O}_3 \rightarrow \text{C}_{20}\text{H}_{20}\text{O}_8\text{N}_8=0.120$ g.). The material was recrystallized from a small volume of pyridine in red needles, M.P. 241° . (Found (Schoeller): C, 47.94, 48.10; H, 4.01, 4.19; N, 22.42, 22.45%. $\text{C}_{20}\text{H}_{20}\text{O}_8\text{N}_8$ requires C, 47.98; H, 4.03; N, 22.39%.)

The compound was almost insoluble in alcohol and gave the violet Neuberg's reaction typical of a bisdinitrophenylhydrazone. Analysis shows, however, that it is the bisdinitrophenylhydrazone of tetrahydroterrein after loss of 1 H_2O . It is later shown that this derivative is identical with the corresponding bis-derivative of the acidic diketone, 4-propyl-2-ketocyclopentanone (XI), obtained by dehydration of tetrahydroterrein on warming with $N \text{H}_2\text{SO}_4$ and also by synthesis. It appears therefore that loss of 1 H_2O has also occurred in the same way during the formation of this bisdinitrophenylhydrazone.

Reaction between tetrahydroterrein and 3:5-dinitrobenzoyl chloride. Tetrahydroterrein (0.15 g.) was kept at room temperature with 3:5-dinitrobenzoyl chloride (0.75 g.) in pyridine (3 ml.). A crystalline benzoate separated which, after repeated crystallization from alcohol, formed long colourless needles, M.P. 116° . (Found (Schoeller): C, 53.87, 53.89; H, 4.27, 4.33; N, 8.71, 8.69%. $\text{C}_{15}\text{H}_{14}\text{O}_7\text{N}_2$ requires C, 53.89; H, 4.22; N, 8.38%.) It is therefore the monobenzoate of $\text{C}_8\text{H}_{12}\text{O}_2$. Hence in the formation of this derivative as in the formation of the bisdinitrophenylhydrazone, 1 H_2O has been lost. It is shown later that this dinitrobenzoate is identical with that of the acidic diketone, 4-propyl-2-ketocyclopentanone (XI), obtained by dehydration of tetrahydroterrein by heating with $N \text{H}_2\text{SO}_4$ and by synthesis.

Dehydration of tetrahydroterrein with $N \text{H}_2\text{SO}_4$ to give an acidic diketone $\text{C}_8\text{H}_{12}\text{O}_2$ (XI) shown to be 4-propyl-2-ketocyclopentanone. Tetrahydroterrein (1 g.) was boiled for 2 hr. under reflux with $N \text{H}_2\text{SO}_4$ (10 ml.). Very soon after boiling commenced, a pale yellow oil separated from solution. After cooling, the product was extracted with ether, the ethereal solution dried over anhydrous

MgSO₄, and after removal of ether a pale yellow mobile oil remained. An attempt was made to dry this oil to constant weight over P₂O₅ *in vacuo*, but as it proved markedly volatile even at room temperature, complete drying was not achieved. (Found (Weiler): C, 66.36; H, 8.86%. C₈H₁₂O₂ requires C, 68.53; H, 8.63%, whilst C₈H₁₄O₃ requires C, 60.72; H, 8.92%.) Examination of these analytical data shows that the reaction has involved a loss of 1 H₂O although the product was not completely dried.

The oil was soluble in alkali, giving a pale yellow colour. 0.0581 g. in 9 ml. alcohol required 3.85 ml. *N*/10 NaOH for neutralization to phenolphthalein corresponding to an equivalent of 151 (C₈H₁₂O₂ titrating as a monobasic acid requires 140). Rotation in alcohol, nil.

The oil gave a very intense red-violet FeCl₃ colour, did not reduce Fehling's solution in the cold and with Brady's reagent gave immediately a thick red precipitate, which after drying melted at 197°. The product was boiled with a little alcohol and the insoluble portion, after recrystallization from nitrobenzene, melted at 241° alone or mixed with the bisdinitrophenylhydrazone from tetrahydroterrein (p. 993) or from the synthetic product. (Found (Weiler): N, 21.92%. C₂₀H₂₀O₈N₈ requires N, 22.39%.) The compound is insoluble in alcohol and gives the violet colour with alcohol-NaOH typical of a bisdinitrophenylhydrazone.

Synthesis of 4-propyl-2-ketocyclopentanone (XI). β -*n*-Propylglutaric acid, m.p. 52°, was first prepared by the method of Day & Thorpe [1920] by condensation of *n*-butaldehyde with cyanoacetamide followed by hydrolysis of the resulting $\alpha\alpha'$ -dicyano- β -*n*-propylglutaramide with boiling dilute HCl. β -*n*-Propylglutaric acid was esterified and condensed with ethyl oxalate in presence of sodium ethoxide using the method of Dickens *et al.* [1922]. Sodium ethoxide (1 g.), prepared in the usual way and heated for 2 hr. in partial vacuum at 200° in an atmosphere of hydrogen, was added to ethyl oxalate (2.2 g.). After a few min., dry ether (10 ml.) and ethyl β -*n*-propylglutarate (3.4 g.) were added, the latter being washed in with dry ether (10 ml.). The mixture was boiled for 24 hr. The brown ethereal solution was cooled in ice, ice-cold 2*N* H₂SO₄ (10 ml.) was slowly added with shaking and the ethereal layer removed, dried over MgSO₄ and evaporated. The residual oil gave a red FeCl₃ colour.

The ester was hydrolysed and decarboxylated, using the method of Baeyer [1894] as employed by Dieckmann [1897], by boiling under reflux for 2 hr. with a solution (30 ml.) obtained by mixing conc. H₂SO₄ (45 ml.) and water (150 ml.). The diketone was extracted with ether, the ethereal extract dried, the ether removed and the material fractionated *in vacuo*. Only a small yield of the diketone resulted and it was present for the most part in the fraction b.p. 135° at 15 mm. It was acid to litmus and gave a red FeCl₃ colour. On emulsifying in water, adding Brady's reagent and keeping overnight, the bisdinitrophenylhydrazone of 4-propyl-2-ketocyclopentanone resulted. After recrystallizing from a large volume of benzene it melted at 241°, not depressed by mixing with

the bisdinitrophenylhydrazone prepared directly from tetrahydroterrein, and gave the same bluish red colour with alcoholic NaOH. (Found (Weiler): C, 48.24, 48.05; H, 4.03, 3.97; N, 22.26, 22.08 %. $C_{20}H_{20}O_8N_8$ requires C, 47.98; H, 4.03; N, 22.39 %.)

Reaction of 3:5-dinitrobenzoyl chloride with 4-propyl-2-ketocyclopentanone (XI). The reaction was carried out as with tetrahydroterrein. Crystals separated which after recrystallization from alcohol melted at 116° and did not depress the m.p. of the 3:5-dinitrobenzoate prepared from tetrahydroterrein. (Found (Schoeller): C, 53.99; H, 4.40; N, 8.46 %. $C_{15}H_{14}O_7N_2$ requires C, 53.89; H, 4.22; N, 8.38 %.) Hence tetrahydroterrein on heating with $N H_2SO_4$ loses 1 H_2O to give 4-propyl-2-ketocyclopentanone (XI) which in the enolized form benzoylates readily.

Reduction of 4-propyl-2-ketocyclopentanone (XI) to give 4-propyl-2-hydroxycyclopentanone (XIII). 4-Propyl-2-ketocyclopentanone (0.35 g.) from tetrahydroterrein in 50 % alcohol (30 ml.) was reduced with hydrogen in presence of a catalyst prepared from $PdCl_2$ (0.2 g.) and charcoal (1 g.). 60 ml. of H_2 were absorbed in 3 min., the reaction then ceasing. (Absorption of 2 atoms of hydrogen corresponds to an uptake of 56 ml. of hydrogen at N.T.P.) The alcoholic solution, after removal of the catalyst gave no colour with $FeCl_3$, reduced Fehling's solution immediately in the cold and with Brady's reagent gave slowly, as with acetoin, a precipitate which at first contained a monodinitrophenylhydrazone, but later became the bis-derivative, 2 hydrogen atoms being lost in the formation. The monodinitrophenylhydrazone, on recrystallization from benzene, separated in fine yellow needles, m.p. 196° . (Found (Weiler): C, 52.80, 52.68; H, 5.71, 5.81; N, 17.72, 17.77 %. $C_{14}H_{18}O_5N_4$ requires C, 52.16; H, 5.63; N, 17.39 %.) The compound gave the red colour with alcohol-NaOH typical of a monodinitrophenylhydrazone and had the same m.p. as, and was isomeric with, the monodinitrophenylhydrazone of 4-propyl-3-hydroxycyclopentanone (X), but gave with it a mixed m.p. depression of 5° . The bisdinitrophenylhydrazone after recrystallization from nitrobenzene-toluene melted at 241° and did not depress the m.p. of the bisdinitrophenylhydrazone prepared directly from tetrahydroterrein or from synthetic 4-propyl-2-ketocyclopentanone.

Dehydration of tetrahydroterrein by distillation. Tetrahydroterrein (2.1 g.) was heated in a small distilling flask in an oil-bath and a slow stream of dry nitrogen passed. At a bath temperature of $130-140^\circ$ water was obviously eliminated but the oily product distilled along with the water. The temperature was raised to 180° and the water and oil collected, yield 1.75 g. Even in a slow stream of nitrogen some loss occurred owing to the considerable volatility. Moreover, owing to the volatility, it was not possible completely to dry the oil even at the ordinary temperature. (Found (Schoeller): C, 66.51; H, 8.69 %. $C_8H_{14}O_3$ requires C, 60.72; H, 8.92; whilst $C_8H_{12}O_2$ requires C, 68.53; H, 8.63 %.) The rise in the carbon content may be taken as sufficient indication of the loss of water. $[\alpha]_{D^{20}}^{25.461} + 19.8^\circ$ ($c = 0.706$ in alcohol). This product, which was an acidic yellow oil, insoluble in water and giving a red-violet $FeCl_3$ colour, was shown to be a mixture of 4-propyl-2-ketocyclopentanone (XI) and chiefly 4-propyl-3-ketocyclopentanone (VII). With Brady's reagent it gave a mixture of two bisdinitrophenylhydrazones, both almost insoluble in alcohol and giving the blue colour with NaOH-alcohol typical of bis-derivatives. They were very difficult to separate, but one of them was shown to be identical with the bisdinitrophenylhydrazone of 4-propyl-2-ketocyclopentanone (XI).

Reduction of distilled tetrahydroterrein. Distilled tetrahydroterrein (0.53 g.) in alcohol (50 ml.) was reduced by shaking with hydrogen and a catalyst prepared from $PdCl_2$ (0.2 g.) and charcoal (1 g.). In 2 min. 92.5 ml. hydrogen were

absorbed, the reaction then ceasing. (An uptake of 2 atoms of hydrogen requires an absorption of 84.8 ml. at N.T.P.) After removal of the catalyst, the alcohol was evaporated *in vacuo* and the product distilled *in vacuo*. A fraction was collected as a colourless oil, B.P. 105–107°/24 mm. (Found (Schoeller): C, 66.95; H, 9.82%. $C_8H_{14}O_2$ requires C, 67.56; H, 9.93%.) This oil was no longer acidic and did not give a $FeCl_3$ colour. With Brady's reagent, it gave chiefly a monodinitrophenylhydrazone, M.P. 194°, which did not depress the M.P. of the monodinitrophenylhydrazone of 4-propyl-3-hydroxycyclopentanone (X), together with a small amount of a bisdinitrophenylhydrazone, M.P. 241°, identical with that of the unreduced material.

Exhaustive reduction of terrein. Formation of 4-propyl-3-hydroxy- and of 4-propyl-2-hydroxy-cyclopentanone (X) and (XIII). When terrein is reduced in aqueous solution with $Pd-C-H_2$ as in the preparation of tetrahydroterrein (p. 992), but for a much longer time, the rapid initial absorption of $2H_2$ is followed by a much slower absorption. Thus terrein (0.2604 g.) with a catalyst prepared from charcoal (1 g.) and $PdCl_2$ (0.2 g.) absorbed 75.7 ml. H_2 in 5 min. at N.T.P. and a further 40.3 ml. in the subsequent 200 min. (Absorption of 1 mol. H_2 requires 37.9 ml.)

In a similar experiment 2 g. terrein were reduced, the catalyst filtered off and both liquid and catalyst were separately extracted continuously with ether. The combined ethereal extracts were dried over anhydrous $MgSO_4$, the ether removed and the resulting oil distilled. A fraction was collected, B.P. 99–101°/5 mm. and was redistilled and collected at 87°/2 mm. (Found (Schoeller): C, 66.95, 66.81; H, 9.91, 9.86%. $C_8H_{14}O_2$ requires C, 67.56; H, 9.93%.) The oil was almost insoluble in water, did not give a colour with $FeCl_3$ but reduced Fehling's solution instantly in the cold. In the formation of this oil from tetrahydroterrein, therefore, 2 atoms of hydrogen had been used and 1 atom of oxygen removed, indicating that a $CHOH$ group had been reduced to CH_2 . As will be seen from the following experiments, this oil consists of a mixture of 4-propyl-2-hydroxy- and 4-propyl-3-hydroxy-cyclopentanones.

Separation of 4-propyl-3-hydroxycyclopentanone (X). Long colourless needles slowly separated from the above oil on standing, which after recrystallization from benzene melted at 124°. (Found (Schoeller): C, 67.65, 67.65; H, 9.88, 9.87%. $C_8H_{14}O_2$ requires C, 67.56; H, 9.93%.) A Zerewitinoff estimation (Roth) gave in anisole 0.99 and 1.1 active H atoms, and in pyridine 1.16 and 1.22 active H atoms at 18 and 95° in each case.

This compound did not reduce Fehling's solution instantly in the cold but only slowly over 48 hr. With Brady's reagent, it gave immediately a yellow monodinitrophenylhydrazone which after recrystallization from benzene or aqueous alcohol melted at 196°. (Found (Schoeller): C, 52.18, 52.22; H, 5.58, 5.63; N, 17.70, 17.55%. $C_{14}H_{18}O_5N_4$ requires C, 52.14; H, 5.63; N, 17.38%.)

The compound, on standing for 1 hr. with semicarbazide hydrochloride, sodium acetate and a few drops of water, readily gave a semicarbazone which after recrystallization from water separated in colourless platelets, M.P. 157°. (Found (Schoeller): C, 54.50, 54.35; H, 8.72, 8.81; N, 21.11%. $C_9H_{17}O_2N_3$ requires C, 54.24; H, 8.61; N, 21.09%.)

The above properties, in particular the lack of reducing power to Fehling's solution and the formation of a monodinitrophenylhydrazone only, agree with the formulation of this compound as 4-propyl-3-hydroxycyclopentanone (X), which has been derived from tetrahydroterrein (III) (4-propyl-2:3-dihydroxycyclopentanone) by conversion of the $CHOH$ group in position 2 into a CH_2 group.

4-Propyl-2-hydroxycyclopentanone (XIII). In the above preparation of crystalline 4-propyl-3-hydroxycyclopentanone, a considerable amount of material remained as an oil and could not be obtained crystalline. The oil contained in addition to a further amount of 4-propyl-3-hydroxycyclopentanone, separable as the monodinitrophenylhydrazone, m.p. 196°, a second substance which reduced Fehling's solution instantly in the cold, and on standing with Brady's reagent, slowly, as with acetoin, gave a bisdinitrophenylhydrazone, 2 atoms of H being lost in the reaction. This bisdinitrophenylhydrazone on recrystallization from nitrobenzene melted at 241°, and did not depress the m.p. of the bisdinitrophenylhydrazone of 4-propyl-2-ketocyclopentanone (XI) either before or after reduction, or of the bisdinitrophenylhydrazone of tetrahydroterrein, 4-propyl-2:3-dihydroxycyclopentanone (III). It seems, therefore, justifiable to assume that the substance giving rise to this bisdinitrophenylhydrazone is 4-propyl-2-hydroxycyclopentanone (XIII), identical with the substance obtained by reduction of 4-propyl-2-ketocyclopentanone (XI).

Exhaustive reduction of tetrahydroterrein. In a further experiment, it was found that crystalline tetrahydroterrein, on reduction, absorbs 1 H₂ at a rate identical with that of the slow second phase of reduction of terrein, and here again crystalline 4-propyl-3-hydroxycyclopentanone, along with an oil, was obtained.

Oxidation experiments with terrein and tetrahydroterrein

(a) *Oxidation of terrein with alkaline iodine.* Terrein (1 g.) was dissolved in water (25 ml.), *N*/10 iodine (390 ml.) and *N* NaOH (60 ml.) were added and the solution kept in the dark at room temperature for 24 hr. A trace of CHI₃ was formed. After acidification, titration with thiosulphate showed an absorption of 4.88 atoms of iodine. The solution was then neutralized to phenolphthalein, evaporated *in vacuo* to a small volume, acidified and extracted continuously with ether. The brown ethereal extract was dried over MgSO₄, and on removal of ether gave a brown oil, from which on rubbing with ethyl acetate and cooling, a crystalline solid separated; colourless needles from ethyl acetate, m.p. 183°. (Found (Schoeller): C, 52.22; H, 4.46%. C₈H₈O₅ requires C, 52.17; H, 4.38%.) 3.277 mg. on neutralization to methyl red required 2.40 ml. *N*/70 NaOH corresponding to an equivalent of 95.6. (C₈H₈O₅ titrating as a dibasic acid requires 92.)

(b) *Oxidation of terrein with periodic acid.* This oxidation was carried out using the modification of the Malaprade method [1928] described by Clutterbuck & Reuter [1935], both the oxygen absorption and the acid production being determined.

(1) *Oxygen absorption.* 0.0586 g. of terrein used an amount of oxygen equivalent to 14.5 ml. *N*/10 iodine. The theoretical value assuming the absorption of 2 atoms of oxygen is 15.2 ml.

(2) *Acid production.* Using 0.5004 g. terrein the acid formed was equivalent to 6.0 ml. *N* NaOH. The production of 2 equiv. of acid corresponds to 6.5 ml. *N* NaOH. The solution resulting from this experiment was then divided into 2 equal parts and used for investigation of the nature of the products.

(i) To half the solution, Brady's reagent was added and a yellow crystalline compound was slowly deposited during 3 weeks. After recrystallization from ethyl acetate it separated in fine radiating, yellow needles, insoluble in NaHCO₃, m.p. (with careful heating) 221°. The m.p. varied considerably with the rate of heating. (Found (Schoeller) on sample dried at 63° *in vacuo*: C, 48.64, 48.84; H, 3.83, 3.84; N, 17.52, 17.55%. C₁₃H₁₂O₆N₄, i.e. monodinitrophenylhydrazone of C₇H₈O₃ requires C, 48.74; H, 3.78; N, 17.50%.)

(ii) The remainder of the solution was treated by the method of Fincke [1913] for the determination of its formic acid content. The Hg_2Cl_2 precipitate weighed 0.616 g. (The theoretical weight of Hg_2Cl_2 corresponding to the production from 0.2502 g. terrein of 1 mol. of formic acid is 0.767 g.) Terrein, therefore, with HIO_4 absorbs 2 atoms of oxygen and gives 1 mol. each of formic acid and of a compound $\text{C}_7\text{H}_8\text{O}_3$.

Isolation and properties of the acid $\text{C}_7\text{H}_8\text{O}_3$ (XXV). Terrein (2 g.) was dissolved in 1% H_2SO_4 (500 ml.) containing KIO_4 (6 g.). The rotation of this fluid immediately after mixing was $\alpha_{5461} + 0.77^\circ$. After 6 hr. at room temperature the rotation had fallen to $+0.10^\circ$ and after 24 hr. to $+0.02^\circ$. The solution was then neutralized to litmus and exhaustively extracted with ether. After removal of ether and drying 1.3 g. of oil remained which crystallized later; colourless prisms *ex benzene*, m.p. 82° . (Found (Schoeller): C, 59.99, 60.00; H, 5.73, 5.81%. $\text{C}_7\text{H}_8\text{O}_3$ requires C, 59.98; H, 5.76%.) Rotation, nil.

In a Zerewitinoff estimation (Roth) it gave, both in anisole and in pyridine, 0.99 and 1.01 mol. CH_4 at 18 and 95° in both cases respectively. 0.0852 g. gave a negligible titration to phenolphthalein in the cold, but on heating on the water-bath for 1 hr. with excess $N/10$ NaOH 6.82 ml. were required for neutralization, corresponding to an equivalent of 125 ($\text{C}_7\text{H}_8\text{O}_3$ titrating as the lactone of a monobasic acid requires 140). The solution during heating developed a yellow colour and some decomposition had obviously taken place.

It did not give a Schiff reaction for a free CHO grouping and with Brady's reagent slowly (over some 3 weeks) gave the same dinitrophenylhydrazone, m.p. 221° , as is described on p. 997. It appears that the presence of a CHO group formed by the action of HIO_4 is masked by ring formation with the COOH group (a reaction of common occurrence with γ -aldehydo-acids), the ring structure being stabilized by the double bond also present (see structure (XXV)). The presence and slow liberation of the CHO group was confirmed by a hydroxylamine titration [Schultes, 1934]. Using 0.0928 g. of $\text{C}_7\text{H}_8\text{O}_3$, 2.7, 3.8, 6.25 and 7.6 ml. $N/10$ NaOH were used in 3, 6, 24, 48 hr. (Theory for 1 CHO group 6.63 ml.)

Catalytic reduction of the acid $\text{C}_7\text{H}_8\text{O}_3$ (XXV) to the lactone of β -propyl- γ -hydroxybutyric acid (XXVI). A solution of the acid $\text{C}_7\text{H}_8\text{O}_3$ (2.35 g.) in water (75 ml.) was shaken with a Pd-norite catalyst prepared as previously described. The equivalent of 1 mol. hydrogen was immediately absorbed, whilst a further 2 mol. were absorbed more slowly, the second taking about 15 min. and the third about 4 hr. The product was no longer soluble in water and was almost entirely adsorbed on the charcoal catalyst. On filtering and extracting the charcoal with ether in a Soxhlet apparatus, an almost theoretical yield of a very mobile colourless oil was obtained which did not crystallize. It was distilled *in vacuo* and practically the whole of the oil distilled at $104\text{--}106^\circ/18$ mm. (Found (Schoeller): C, 64.45, 64.30; H, 9.87, 9.93%. $\text{C}_7\text{H}_{12}\text{O}_2$ requires C, 65.58; H, 9.45%.)

0.0689 g. of this oil gave a negligible titration in the cold, but on heating with excess $N/10$ NaOH for 1 hr. and back-titrating to phenolphthalein required 5.42 ml. $N/10$ NaOH corresponding to equiv. wt. 127 ($\text{C}_7\text{H}_{12}\text{O}_2$ titrating as the lactone of a monobasic acid requires 128). The oil no longer reduced Fehling's solution and did not give a dinitrophenylhydrazone with Brady's reagent. It gave, however, on warming on the water-bath with a slight excess of redistilled phenylhydrazine and a little alcohol, a beautifully crystalline, almost colourless phenylhydrazone which after recrystallization from ether-light petroleum separated in plates, m.p. 115° alone or mixed with a synthetic specimen of the phenylhydrazone of β -propyl- γ -hydroxybutyric acid (see p. 999). (Found (Schoeller):

C, 66.18, 66.29; H, 8.52, 8.53; N, 11.98, 11.83 %. $C_{13}H_{20}O_2N_2$ requires C, 66.06; H, 8.54; N, 11.86 %.)

The oil on oxidation gave rise to *dl*-propylsuccinic acid (VI). The oil (0.1 g.) was added to Kiliani mixture (2 ml.), and kept for a week at room temperature with frequent shaking. The oily layer had then set solid and was filtered off. After recrystallization from light petroleum (B.P. 80–100°) it melted at 94°, not depressed by mixing with a synthetic sample of *dl*-propylsuccinic acid (see p. 1000).

Synthesis of β -n-propylbutyrolactone (XXVI). β -n-Propylglutaric acid, prepared as described on p. 994, was converted into the NH_4 salt, which on heating in an oil-bath at 190–200° readily lost NH_3 and gave β -propylglutarimide which crystallized from alcohol in shining plates, m.p. 112°. (Found (Weiler): C, 61.61, 61.82; H, 8.35, 8.51; N, 8.85, 8.96 %. $C_8H_{13}O_2N$ requires C, 61.90; H, 8.45; N, 9.03 %.) The Hoffmann degradation with the formation of β -propyl- γ -aminobutyric acid was then carried out using both NaOBr and NaOCl.¹ The yield was extremely poor with NaOBr and moderate with NaOCl. The imide (3.2 g.) was first brought into solution by heating and stirring with water (150 ml.) containing NaOH (0.3 g.); the liquid was cooled to 0°, a solution of 10 % NaOH (10.1 g.) and 5 % NaOBr (28.8 g.) (or 9 g. of 10 % NaOCl) was added, kept 15 min. and then heated 15 min. A few drops of bisulphite were added and the solution made acid with HCl.

The aqueous solution of the amino-acid hydrochloride, in order to obtain the corresponding hydroxy-compound, was treated with a suspension of $AgNO_3$, the $AgCl$ filtered off and the filtrate heated until evolution of N_2 ceased; the solution was then made alkaline, evaporated to a small bulk and acidified. The resulting oil was extracted with ether, the ethereal solution dried, the ether removed and the oil fractionated. The lactone was collected, B.P. 110–112°/20 mm.

Phenylhydrazide. The lactone was converted into the phenylhydrazide by the method used by Clutterbuck *et al.* [1935] with butyrolactone. The hydrazide was obtained solid by rubbing with ether-light petroleum and crystallized from benzene in colourless plates, m.p. 115°. It did not depress the m.p. of the corresponding compound, m.p. 115°, obtained from terrein. (Found (Schoeller): C, 65.91; H, 8.44; N, 12.00 %. $C_{13}H_{20}O_2N_2$ requires C, 66.06; H, 8.54; N, 11.86 %.)

(c) *Oxidation of tetrahydroterrein with periodic acid.* The experimental conditions were those described by Clutterbuck & Reuter [1935]. 0.0543 g. of tetrahydroterrein, m.p. 84°, used an amount of oxygen equivalent to 13.70 ml. $N/10$ iodine. The theoretical value assuming the absorption of 2 atoms of oxygen is 13.74 ml.

Brady's reagent was then added to this solution and a yellow precipitate separated which after several recrystallizations from aqueous alcohol was obtained in yellow needles, m.p. 157°. It was soluble in $NaHCO_3$ solution and gave the red colour with alcohol and NaOH typical of a monodinitrophenylhydrazone. (Found (Schoeller): C, 48.15, 48.15; H, 4.85, 4.88; N, 17.23, 17.34 %. $C_{13}H_{16}O_6N_4$ requires C, 48.14; H, 4.98; N, 17.28 %.) The compound is therefore the monodinitrophenylhydrazone of a substance $C_7H_{12}O_3$. On standing in the cold with alcohol- H_2SO_4 it readily gave a compound insoluble in $NaHCO_3$ solution which after recrystallization from aqueous alcohol separated in yellow needles, m.p. 86°. (Found (Schoeller): C, 51.45, 51.27; H, 5.78, 5.74; N, 16.19, 15.84 %. $C_{15}H_{20}O_6N_4$ requires C, 51.12; H, 5.73; N, 15.91 %.) The analysis and the

¹ The suggestion that the synthesis might be effected by a Hoffmann degradation of propylglutaric acid was made by Prof. R. Robinson, and the actual working out of the synthesis was done by Dr W. Koerber, to both of whom we tender our best thanks.

insolubility in NaHCO_3 suggest that esterification of a carboxyl group has occurred.

One product of the oxidation of tetrahydroterrein by HIO_4 is therefore the aldehydo-acid $\text{C}_7\text{H}_{12}\text{O}_3$ (II) and from the structure assigned to tetrahydroterrein (III) this compound must be β -formyl-*n*-hexanoic acid, i.e. one form of the half aldehyde of propylsuccinic acid. This view is later supported by the quantitative oxidation of $\text{C}_7\text{H}_{12}\text{O}_3$ by alkaline iodine to *d-n*-propylsuccinic acid (VI) see below).

In a further similar experiment the presence of formic acid in the oxidation solution was detected and its amount determined by the method of Fincke [1913]. Tetrahydroterrein (0.300 g.) gave 0.779 g. Hg_2Cl_2 . (Theory for the production of 1 mol. formic acid is 0.896 g.) The reaction of tetrahydroterrein with HIO_4 therefore follows the equation

(d) *Oxidation of tetrahydroterrein with periodic acid followed by alkaline iodine. Formation of d-n-propylsuccinic acid (VI).* Tetrahydroterrein (0.250 g.) was dissolved in the usual KIO_4 solution (275 ml.) and 2*N* H_2SO_4 (30 ml.) was added. Next day, KI (6 g.) and 2*N* NaOH (50 ml.) were added and the solution kept for 24 hr. Acidification and titration with *N*/10 thiosulphate revealed that 99.0 ml. *N*/10 iodine had been absorbed. The theoretical amount of iodine, assuming the absorption of 3 atoms of oxygen, is 94.8 ml. The solution was then neutralized, concentrated *in vacuo* to a small bulk, acidified and extracted continuously with ether. The ethereal extract after drying over anhydrous MgSO_4 and removal of solvent gave a brown oil which readily solidified on rubbing with a little light petroleum, B.P. 40/50°. After several recrystallizations from light petroleum, B.P. 80/100° it separated in colourless needles, M.P. 103°, $[\alpha]_{5461}^{18} + 26.6^\circ$ ($c = 0.602$ in H_2O). (Found (Schoeller): C, 52.41; H, 7.50 %. $\text{C}_7\text{H}_{12}\text{O}_4$ requires C, 52.48; H, 7.55 %.) 0.0119 g. required 1.43 ml. *N*/10 alkali for neutralization to phenolphthalein giving equiv. wt. 83.2. $\text{C}_7\text{H}_{12}\text{O}_4$ titrating as a dibasic acid requires 80.

The acid on heating in 50 % KOH for 6 hr. under reflux, and re-isolating, showed no material alteration in rotation, racemization being extremely difficult to effect.

The acid depressed the M.P. of *d-isopropylsuccinic acid*, M.P. 94° (kindly supplied by Drs Henry & Paget [1928]) by 15°; it depressed the M.P. of *dl-αα'*-methylethylsuccinic acid (kindly supplied by Prof. Thunberg) by 30°; it depressed the M.P. of a sample of *dl-αα'*-methylethylsuccinic acid synthesized by the method of Higson & Thorpe [1906] by 25°, but did not depress the M.P. of a sample of *d-n*-propylsuccinic acid, M.P. 98°, $[\alpha] + 9.6^\circ$ kindly supplied by Prof. Timmermans. Since the acid from tetrahydroterrein had, however, a higher M.P. (103°) and rotation, $[\alpha]_{5461}^{18} + 26.6^\circ$, it became necessary to synthesize *dl-n*-propylsuccinic acid and to resolve it.

Synthesis of dl-n-propylsuccinic acid. The synthesis was first effected by condensation of butaldehyde with sodium cyanoacetate and conversion of the α -cyanoacrylic acid into propylsuccinic acid using the general method of Lapworth & McCrae [1922] as carried out by Timmermans & van der Haegen [1933]. The yield obtained was small.

A second synthesis, with larger yield, was effected by condensation of ethyl chloroacetate with ethyl sodiomalonate giving ethyl ethenyltricarboxylate, B.P. 162–165°/20 mm. [Bischoff, 1882], followed by further condensation with *n*-propyl iodide in presence of sodium ethoxide. This reaction was best carried

out in boiling toluene. The resulting ethyl *n*-propylcarboxysuccinate, B.P. 175–178°/28 mm., was obtained in 72% yield. The free acid was obtained by boiling with alcoholic KOH, removing the alcohol and extracting continuously with ether. The crude propylcarboxysuccinic acid was then converted into *dl-n*-propylsuccinic acid by thermal decarboxylation. The best method of purification was by fractional distillation *in vacuo* of the anhydride (B.P. 145–146°/25 mm.). The free acid, obtained from the anhydride, was recrystallized several times from water (2–3 vol.). The m.p. of *dl-n*-propylsuccinic acid obtained by both synthetic methods was eventually raised to 94° but could not be obtained higher. 0.0598 g. of the synthetic acid required 7.4 ml. *N*/10 NaOH for neutralization to phenolphthalein giving equiv. wt. 80.8 ($C_7H_{12}O_4$ titrating as a dibasic acid requires 80).

Resolution of synthetic dl-n-propylsuccinic acid. The resolution was effected by strychnine using the method of Braun & Reinhard [1929]. The acid (7.5 g.) was dissolved in water (450 ml.) and strychnine (31.2 g.) was added, the solution warmed on the water-bath and kept overnight in the cold room. The precipitate was dissolved in water (1500 ml.) at 80–90°, filtered from dissociated strychnine NH_4OH (150 ml.; sp. gr. 0.88) added. The strychnine separating was filtered and the filtrate was evaporated to a small volume, acidified and extracted continuously with ether. 3.55 g. of acid were obtained, m.p. 95–97°, unchanged by recrystallization from light petroleum (B.P. 80–90°), $[\alpha]_{5461}^{18} + 20.6^\circ$ ($c = 1.204$ in H_2O). The resolution was repeated. The whole of the material was dissolved in warm water (280 ml.), strychnine (14.8 g.) was added and the propylsuccinic acid isolated from the precipitate as before. After recrystallization from light petroleum it melted at 103° and did not depress the m.p. of the natural product, m.p. 103°, $[\alpha]_{5461}^{18} + 26.8^\circ$ ($c = 1.12$ in water). (Found (Weiler): C, 52.67, 52.60; H, 7.51, 7.41%. $C_7H_{12}O_4$ requires C, 52.48; H, 7.55%.) 0.0691 g. required 8.65 ml. *N*/10 NaOH for neutralization to phenolphthalein giving equiv. wt. 79.9 ($C_7H_{12}O_4$ titrating as a dibasic acid requires 80).

(e) *Oxidation of tetrahydroterrein with chromic acid.* Oxidation of tetrahydroterrein with Kiliani's chromic acid mixture [Kiliani & Merk, 1901] gave a good yield of *d-n*-propylsuccinic acid, m.p. 100°.

(f) *Oxidation of distilled tetrahydroterrein.* Distilled tetrahydroterrein (see p. 995) was oxidized with $KMnO_4$ in acetone. The product of oxidation proved to be *d-n*-propylsuccinic acid, mixed m.p. 99°. (Found (Schoeller): C, 52.71; H, 7.55%. $C_7H_{12}O_4$ requires C, 52.48; H, 7.55%.)

(g) *Treatment of terrein with ozone.* Ozonized air was passed for 2 hr. into a solution of terrein (0.5 g.) in water (10 ml.), cooled in ice. Nitrogen was then bubbled through the solution and the gases evolved on warming were passed into bubblers containing 2:4-dinitrophenylhydrazine reagent. A copious precipitate of acetaldehydedinitrophenylhydrazone separated which after recrystallization from alcohol separated in small yellow plates, m.p. 163°, and did not depress the m.p. of an authentic specimen.

(h) *γ -Lactone of α -hydroxy- β -*n*-propylglutaric acid, $C_8H_{12}O_4$ (IX).* 2 g. of crude 4-propyl-3-hydroxycyclopentanone (X) were heated with 40 ml. of HNO_3 (sp. gr. 1.2) in a water-bath, the temperature being slowly raised until at 75–80° a vigorous reaction just began. The heating was continued at this temperature for half an hour when evolution of brown fumes apparently ceased. The pale yellow oxidation solution was neutralized to litmus with NaOH and solid $AgNO_3$ added until no further precipitation occurred. The Ag salt was immediately filtered off, rapidly washed with a little water, suspended in dilute H_2SO_4 and the acids recovered by continuous ether extraction. The extracted

material, after removal of solvent, was neutralized, made faintly acid with dilute HCl and a little oxalic acid removed as calcium oxalate. The filtrate was acidified and re-extracted continuously with ether. The ethereal extract was dried over anhydrous MgSO_4 and on evaporation left an oil which solidified on standing *in vacuo* over H_2SO_4 . After several recrystallizations from benzene, it separated in long colourless needles, m.p. 96° . (Found (Schoeller): C, 55.75, 55.59; H, 6.97, 7.10 %. $\text{C}_8\text{H}_{12}\text{O}_4$ requires C, 55.80; H, 7.03 %.) 0.0165 g. required for neutralization to phenolphthalein at room temperature 1.03 ml. *N*/10 NaOH, and after heating with excess NaOH and back-titrating an additional 0.97 ml. *N*/10 NaOH, corresponding to equivalents of 160 and 83 respectively. $\text{C}_8\text{H}_{12}\text{O}_4$, titrating as a mono- and a di-basic acid, requires 172 and 86 respectively. The acid was recovered from the titrated solution and shown by mixed m.p. to be unchanged.

Active hydrogen determinations (Zerewitinoff-Roth) in pyridine and in anisole at 18 and 95° gave 1.00 and 1.06 active H atoms.

On oxidation with Kiliani's mixture it gave *d*-*n*-propylsuccinic acid.

The above analytical data indicate that the compound $\text{C}_8\text{H}_{12}\text{O}_4$ is the γ -lactone of a hydroxylated dibasic acid, and from the structure assigned to the parent substance it is most probably the γ -lactone of α -hydroxy- β -*n*-propylglutaric acid (IX), thus drawing it into line with the structure of propylbutyrolactone (XXVI) obtained from terrein by HIO_4 oxidation and prolonged reduction of the product $\text{C}_7\text{H}_8\text{O}_3$ (XXV).

SUMMARY

The molecular constitution of terrein, $\text{C}_8\text{H}_{10}\text{O}_3$, a metabolic product of certain strains of the group species *Aspergillus terreus* Thom, has been investigated. By a study of its general properties, derivatives and breakdown products it has been shown to be 4-propenyl-2-hydroxy-3:5-oxidocyclopentane-1-one.

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