Constituents of the Lipids of Tubercle Bacilli. Part VI. Phthiocerol.*

By J. A. Hall, J. W. Lewis, and N. Polgar.

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Phthiocerol, a lævorotatory alcohol from the lipids of tubercle bacilli, is shown to afford a benzylidene derivative, thus indicating a 1 : 2- or 1 : 3-glycol structure. Oxidative degradation yields *n*-tetracosanoic acid and a C_{11} methoxy-acid, and the main structural features of phthiocerol in the light of these findings (cf. Hall and Polgar, *Chem. and Ind.*, 1954, 1293, where a preliminary account was given) are discussed. The results of further studies of phthiocerol, including debromination of its dibromo-derivative, and a mild oxidation giving rise to a β-diketone, disclose the presence of a 1 : 3-diol system, and evidence is presented which indicates that a methyl and a methoxyl group are both attached at $C_{(4)}$. It is concluded that phthiocerol is 4-methoxy-4-methyl-*n*-tetratriacontane-9 : 11-diol (V).

Phthiocerol, $[\alpha]_D$ −4·8°, m. p. 73—74°, isolated from the lipids of human and bovine tubercle bacilli (Stodola and Anderson, *J. Biol. Chem.*, 1936, **114**, 467; Reeves and Anderson, *ibid.*, 1937, **119**, 535; Cason and Anderson, *ibid.*, p. 549), was shown by the first-named authors to have the empirical formula $C_{35}H_{72}O_3$ or $C_{36}H_{74}O_3$, and to contain two hydroxyl and one methoxyl group. Contrary to earlier statements (cf. Reeves and Anderson, *J. Amer. Chem. Soc.*, 1937, **59**, 858), the same alcohol was recently found in the lipids of avian tubercle bacilli (Hall and Polgar, unpublished work); it is probably essentially the " phtioglycol " of Stendal (*Compt. rend.*, 1934, **198**, 1549). From the properties exhibited by monolayers of phthiocerol Ställberg and Stenhagen (*J. Biol Chem.*, 1942, **143**, 171) inferred that it contains a long carbon chain with one or more of the polar groups near one end. Reaction of phthiocerol with hydriodic acid and reduction of the resulting iodo-derivative with zinc and acetic acid gave a product which was partly unsaturated and on catalytic hydrogenation, followed by treatment with hot concentrated sulphuric acid, afforded a saturated hydrocarbon, phthiocerane (Stodola and Anderson, *loc. cit.*; Ginger and Anderson, *J. Biol. Chem.*,

* The paper by Marks and Polgar, *J.*, 1955, 3851, is regarded as Part V.

1945, **157**, 213), containing, according to infrared studies, a methyl branch at $C_{(4)}$ (Ställberg-Stenhagen, Stenhagen, Sheppard, Sutherland, and Walsh, *Nature*, 1947, **160**, 580). From a comparison of its m. p. and *X*-ray spacings with those of synthetic hydrocarbons Ställberg-Stenhagen and Stenhagen (*J. Biol. Chem.*, 1948, **173**, 383; 1950, **183**, 223) concluded that phthiocerane is essentially racemic 4-methyltritriacontane, possibly containing small amounts of the optically active form, or of a higher homologue; they interpreted the formation of the racemic product as indicating that one of the functional groups of phthiocerol is attached at the carbon atom bearing the methyl group, or at an adjacent carbon atom.

In the present work phthiocerol, $[\alpha]_D$ −4·45°, m. p. 71·5—73°, was obtained from the lipids of human tubercle bacilli by a modification of the procedure described earlier (Chanley and Polgar, *J.*, 1954, 1003). Its infrared spectrum showed a band at 1712 cm.$^{-1}$ (CO), but purification of the product with the aid of Girard's T reagent indicated that ketonic material was only present as a minor contaminant. It has been claimed (Demarteau-Ginsburg, Ginsburg, and Lederer, *Biochim. Biophys. Acta*, 1953, **12**, 587) that phthiocerol, isolated by the procedure described by Anderson and his collaborators (*loc. cit.*, 1936), is accompanied by two ketones and a triol. Examination of our specimen by chromatography on alumina indicated that, if any triol was present, it could have been only a very small amount.

As already briefly reported (Hall and Polgar, *Chem. and Ind.*, 1954, 1293), phthiocerol was found to yield a benzylidene derivative, thus indicating the presence of a 1 : 2- or 1 : 3-glycol system; moreover, it afforded on oxidative degradation a liquid methoxy-acid, $C_{11}H_{22}O_3$, characterised through its *S*-benzylthiuronium salt, m. p. 137—139°, together with an acid, m. p. 76—77°, having the composition $C_{24}H_{48}O_2$ or a near homologue. We are grateful to Mrs. D. M. Hodgkin, F.R.S., for comparing the *X*-ray powder photograph of the latter acid with those of authentic specimens of *n*-tricosanoic and *n*-tetracosanoic acid (kindly provided by Dr. J. C. Smith). Mrs. Hodgkin reported as follows:

" The powder photographs of tetracosanoic acid and the degradation product seem to be essentially identical. They both show powder lines at the spacings 3·8, 4·1, 4·6, 11, 20, and 35 Å, but several of these lines seem to differ slightly in intensities. The powder photograph of tricosanoic acid shows powder lines at spacings of 3·8, 4·1, 4·7, 12, and 20 Å, but no line appears at 35 Å, while two additional lines appear at 3·7 and 18 Å."

On the assumption that the C_{11} methoxy-acid is a homogeneous substance, the above results show that phthiocerol has the structure (I) or (II) of which only (I) is compatible with the C_{34} formula proposed for the parent hydrocarbon (see above). In attempting to distinguish between these alternatives by examining the action of periodic acid on phthiocerol we found that a slow oxidation had taken place, and the product showed in its infrared spectrum a strong band at 1712 cm.$^{-1}$ (CO). In the earlier phases of this work (cf. Hall and Polgar, *loc. cit.*) we were, therefore, in favour of formula (I), but this view had to be revised in the light of the following evidence.

(I) $CH_3 \cdot [CH_2]_{22} \cdot CH(OH) \cdot CH(OH) \cdot C_9H_{18}(OMe)$

(II) $CH_3 \cdot [CH_2]_{22} \cdot CH(OH) \cdot CH_2 \cdot CH(OH) \cdot C_9H_{18}(OMe)$

Reaction of phthiocerol with hydrogen bromide in glacial acetic acid at room temperature gave essentially a dibromide which, by the action of zinc and sodium iodide in acetone, afforded a bromine-free product. The latter, on oxidation with potassium permanganate in acetone, was found to contain only traces of olefinic material, probably formed by partial elimination of the methoxyl group. Since on debromination of a 1 : 2-dibromide an olefin should arise, these experiments indicated that phthiocerol is not a 1 : 2-diol, and the above bromine-free product must be largely a *cyclo*propane derivative resulting from the 1 : 3-diol (II) *via* the corresponding 1 : 3-dibromide. It should be noted that the infra-red spectrum of the dibromide showed, in addition to a weak band at 961·5 cm.$^{-1}$ (*trans*-CH : CH-), a moderate band at 1739 cm.$^{-1}$ (ester-carbonyl), probably due to the presence of a diacetoxy-derivative, a certain quantity of which may be expected to arise by the action of hydrogen bromide in acetic acid (cf. Stoll and Commarmont, *Helv. Chim. Acta*, 1948, **31**, 1077); however, judged from the analytical data for the debrominated material, the diacetoxy-derivative must have been a minor by-product.

A further proof for the 1 : 3-diol structure of phthiocerol was obtained by oxidation with a very dilute anhydrous solution of chromium trioxide in glacial acetic acid. The product, as shown by its ultraviolet and infrared spectra and a positive ferric chloride test (see Experimental section), consisted of a β-diketone and an αβ-unsaturated ketone, together with unchanged phthiocerol, and acidic oxidation products. A complete separation of the latter from the non-acid constituents was found in earlier experiments to involve considerable difficulties, and it should be pointed out that only traces of β-diketone resulted if water was added on dissolving the chromium trioxide in acetic acid.*

If the structure (II), representing phthiocerol as $C_{36}H_{74}O_3$, is accepted, it follows that the parent hydrocarbon must be $C_{35}H_{72}$. This conclusion conflicts with the view of Ställberg-Stenhagen and Stenhagen regarding the formula of phthiocerane (see above), but taking into account the 1 : 3-diol structure of phthiocerol it appears possible that in the procedure employed for transforming the latter into phthiocerane side-reactions may have occurred resulting in a mixture of isomers. Thus, the intermediate iodo-derivative, being a 1 : 3-di-iodide, may have given rise, by the action of zinc, to a *cyclo*propane derivative as a by-product; conversion of the latter into methyl-branched isomers could then have occurred during the subsequent catalytic hydrogenation. We believe, therefore, that the C_{36} formula for phthiocerol is not ruled out by the experimental evidence, namely, the *X*-ray and thermal data, recorded for phthiocerane (the infrared spectra did not differentiate between 4-methyltritriacontane and 4-methyltetratriacontane), but a final decision on this point must await the identification of the C_{11} oxidation product described above.

In further experiments phthiocerol was refluxed with a solution of hydrogen bromide in glacial acetic acid, and the resulting bromo-derivative debrominated with zinc and sodium iodide in acetone, to give an unsaturated hydrocarbon which gave correct analyses for $C_{35}H_{68}$ (when hydriodic acid was used instead of hydrogen bromide in glacial acetic acid, de-iodination of the product with zinc resulted in a hydrocarbon containing only a small proportion of unsaturated material.) Ozonisation of the unsaturated hydrocarbon afforded, in addition to non-volatile material presumed to be essentially a C_{30} acid and a C_{32} ketone, volatile products which were isolated as their 2 : 4-dinitrophenylhydrazones. Paper chromatography, kindly carried out by Dr. H. S. Burton, indicated the presence of the 2 : 4-dinitrophenylhydrazones of propaldehyde and methyl propyl ketone. These results suggested that the above unsaturated hydrocarbon was probably a mixture of the isomers (III) and (IV). It then follows that in phthiocerol presumably both the methoxyl and a methyl group are attached at $C_{(4)}$ as in (V). This conclusion is consistent with the earlier view (see above) that phthiocerane is essentially a racemic 4-methyl-substituted hydrocarbon.

(III) $CH_3\cdot[CH_2]_{22}\cdot\underset{\diagdown\,\diagup}{CH}(CH_2)\cdot CH\cdot[CH_2]_3\cdot CH{:}CMe\cdot[CH_2]_2\cdot CH_3$

(IV) $CH_3\cdot[CH_2]_{22}\cdot\underset{\diagdown\,\diagup}{CH}(CH_2)\cdot CH\cdot[CH_2]_4\cdot CMe{:}CH\cdot CH_2\cdot CH_3$

(V) $CH_3\cdot[CH_2]_{22}\cdot CH(OH)\cdot CH_2\cdot CH(OH)\cdot[CH_2]_4\cdot CMe(OMe)\cdot[CH_2]_2\cdot CH_3$

Demarteau-Ginsburg and Lederer (*Compt. rend.*, 1955, **240**, 815) recently suggested that the methoxyl group is at $C_{(5)}$, and proposed formula (VI) for phthiocerol. This is incompatible with the results of our ozonisation experiments, since neither propaldehyde

(VI) $CH_3\cdot[CH_2]_{22}\cdot CH(OH)\cdot CH_2\cdot CH(OH)\cdot[CH_2]_2\cdot CH(OMe)\cdot CHMe\cdot[CH_2]_2\cdot CH_3$

nor a non-volatile ketone could have resulted from structure (VI) by the procedure described; moreover, it does not account for the formation of a C_{11} methoxy-acid on oxidation of phthiocerol.

* During the later stages of this work, after the appearance of our preliminary note, Dr. Frank H. Stodola kindly placed at our disposal some unpublished results of studies made twenty years ago in Professor R. J. Anderson's laboratory, which involved oxidation of phthiocerol with chromic acid, and, judging from the high consumption of chromic acid and the liberation of over a mol. of carbon dioxide in this process, he concluded that phthiocerol probably has a 1 : 3-diol structure.

Experimental

All spectra were determined by Dr. F. B. Strauss with the technical assistance of Mr. F. Hastings; ultraviolet spectra were determined for MeOH solutions.

Isolation of Phthiocerol.—The procedure described in an earlier communication (Chanley and Polgar, *loc. cit.*) was followed with the following modifications. The methanol-insoluble product (about 500 g.) resulting from the partial hydrolysis of the lipids of tubercle bacilli (human type), dissolved in benzene (2·5 l.), was refluxed with potassium hydroxide (200 g.) in methanol (1 l.) for 200 hr., and the hot solution then filtered. In contrast to the earlier procedures which omitted this filtration, no emulsions were encountered in the subsequent operations involving acidification (dilute hydrochloric acid), ether-extraction, and washing of the combined extracts with water. After evaporation of the dried ($MgSO_4$) benzene–ether extracts, the residue (460 g.) was dissolved in benzene (2 l.), and methanol (5 l.) was added with shaking. Next day the precipitate (" mycolic acid;" 270 g.) was removed by filtration and washed with a mixture of benzene (200 c.c.) and methanol (500 c.c.); the combined filtrate and washings were evaporated. The residual product (190 g.) was dissolved in benzene (750 c.c.), and a solution of barium hydroxide (90 g.) in hot methanol (1 l.) added with stirring; the mixture was heated to boiling, and then kept at room temperature. Next day the solution was decanted, filtered (to remove insoluble barium salts), and concentrated (to about 1 l.), and the residual solution was poured into dilute hydrochloric acid. The aqueous layer was extracted with ether, and the ethereal extract, together with the benzene layer, washed with dilute hydrochloric acid, water, and dried ($MgSO_4$). After removal of the solvents, the residue (92 g.) was dissolved in boiling light petroleum (b. p. 40—60°; 500 c.c.). When the solution was kept overnight, a solid separated which after recrystallisation from ethyl acetate gave phthiocerol (32·5 g.) as colourless plates, m. p. 71·5—73°, $[\alpha]_D$ −4·45° (*c*, 11·48 in $CHCl_3$) (Found: C, 77·6; H, 13·4. Calc. for $C_{36}H_{74}O_3$: C, 78·0; H, 13·4%); its acetate, prepared by refluxing phthiocerol with acetic anhydride, distilled at 270—280° (bath)/0·04 mm. (Found: C, 75·15; H, 12·2. Calc. for $C_{40}H_{78}O_5$: C, 75·2; H, 12·2%).

The above petroleum mother-liquors from which the crude phthiocerol separated were found, on chromatography over alumina, to contain, largely, acidic material. Some earlier specimens of phthiocerol might have been contaminated with such acids.

Purification of Phthiocerol by Treatment with Girard-T Reagent.—Phthiocerol (1·14 g.) was refluxed with ethanol (50 c.c.), glacial acetic acid (5 c.c.), and Girard-T reagent (3 g.) for 1 hr., and the mixture then kept at room temperature overnight. On working up in the known manner (addition to ice-cold aqueous potassium hydroxide, ether-extraction) a " ketone " fraction (0·11 g.) resulted, the infrared spectrum of which showed a band at 1712 $cm.^{-1}$ (CO); this was absent in case of the " non-ketone " fraction (1 g.). It should be noted that during the above ether extractions and on washing the ethereal extracts with water, emulsions resulted which separated partly on standing, but complete separations of the organic and aqueous phases could not be achieved.

When 5 g. of phthiocerol were used, the " ketone " fraction resulting from the above procedure amounted to 1 g. The combined " ketonic " fractions (1·11 g.), when subjected to a repetition of the Girard separation, yielded 0·56 g. of " ketone." This was chromatographed in benzene (50 c.c.) on neutral alumina (activity II on the scale of Brockmann and Schodder, *Ber.*, 1941, **74**, 73) (15 g.; 1·4 × 9·5 cm.) prepared in benzene. The following fractions were obtained: (i) 0·005 g. eluted by benzene, (ii) 0·11 g. eluted by benzene–ether (4 : 1), (iii) 0·113 g. eluted by benzene–ether (7 : 3), (iv) 0·071 g. eluted by benzene–ether (3 : 2), (v) 0·081 g. eluted by ether, and (vi) 0·112 g. eluted by ether–methanol (19 : 1). The fraction (vi), which showed the strongest band at 1712 $cm.^{-1}$, was refluxed with ethanol (10 c.c.), hydroxylamine hydrochloride (0·1 g.), and sodium acetate (0·1 g.) for 5 hr., and, after dilution with water, the product was isolated by ether-extraction. For analysis the crude product was dried *in vacuo* at 100° for 1 hr. (Found: N, 2·3, 2·0%).

The above " non-ketone " fraction was chromatographed in benzene–ether (1 : 1) on neutral alumina (activity II), and the column eluted with the same solvent. Further elution with ether–methanol (19 : 1) removed small amounts of tenaciously held material which was rechromatographed under the same conditions, and the resulting ether–methanol eluate was subjected to a repetition of this procedure. The product finally eluted with ether–methanol had m. p. 70—75° after crystallisation from ethyl acetate and showed a medium band at 1093 $cm.^{-1}$ (aliphatic ether group); it represented about 0·6% of the original phthiocerol, and in view of the small amounts available was not investigated further.

Benzylidenephthiocerol.—A mixture of phthiocerol (0·2 g.), benzaldehyde (5 c.c.), and powdered anhydrous zinc chloride (1 g.) was shaken at room temperature for 12 hr. and set aside for a further 48 hr. Ether (50 c.c.) was then added and the solution shaken with anhydrous sodium carbonate (1 g.) for 1 hr. After filtration, the ethereal solution was washed successively with 5% aqueous sodium carbonate and water, then dried (Na_2SO_4) and evaporated. Trituration of the residue with methanol, followed by crystallisation of the separated solid from methanol, gave O-*benzylidenephthiocerol* as a colourless solid, m. p. 34—38° (Found : C, 80·4; H, 12·5. $C_{43}H_{78}O_3$ requires C, 80·4; H, 12·2%).

Oxidation of Phthiocerol with Periodic Acid.—Phthiocerol (1 g.) was dissolved in dioxan (100 c.c.), and a solution of periodic acid (1 g.) in water (5 c.c.) added; the resulting solution was kept at room temperature for about 3 weeks, then evaporated under reduced pressure at <30°, and the residue, after addition of water, isolated by ether-extraction. The product, which showed a strong band at 1712 cm.$^{-1}$ (CO) in its infrared spectrum, was chromatographed in benzene on alumina (activity I—II; 30 g.). Two main fractions were obtained, *viz.*, (i) a colourless solid (0·31 g.) eluted by benzene, and (ii) unchanged starting material, m. p. 72—73° (from acetone), eluted by ether–methanol. Fraction (i), after crystallisation from methanol, had m. p. 46—49° (sintering at *ca.* 43°) (Found : C, 80·2; H, 13·1%); an attempt was made to convert the crude material into a semicarbazone, but the product was shown on analysis to contain only a small amount of nitrogen (Found : N, 3·2%). Oxidation with a suspension of silver oxide in ethanolic sodium hydroxide gave small amounts of an acid which was not examined further.

Oxidation of Phthiocerol with Chromic Acid.—A solution of chromic acid (from 1 g. of chromium trioxide, 1 c.c. of water, and 10 c.c. of glacial acetic acid) was added dropwise to phthiocerol (0·5 g.) in glacial acetic acid (50 c.c.), kept at 25—30°, until no more chromic acid was consumed. After 1 hr. at the room temperature the mixture was poured into water, and the product isolated by ether-extraction. The crude material, combined with another batch which was obtained by a similar procedure (together 2 g.), was heated with glacial acetic acid (35 c.c.) and hydrogen peroxide (3 c.c.; 30%) on a steam-bath for 6 hr., a further portion of hydrogen peroxide (3 c.c.) being added after 3 hours' heating. Most of the acetic acid was then removed under reduced pressure, and the residue diluted with water and extracted with ether, from which the acidic fraction was removed by shaking with 2% aqueous potassium hydroxide (3 × 750 c.c.). Acidification of the alkaline extracts with hydrochloric acid, followed by ether-extraction and distillation, gave two acid fractions, *viz.*, (i) b. p. about 160° (bath)/40 mm., and (ii) b. p. 140° (bath)/0·1 mm. Fraction (i) (Found : C, 65·5; H, 10·7. Calc. for $C_{11}H_{22}O_3$: C, 65·3; H, 11·0%) afforded a *S*-benzylthiuronium salt which crystallised from aqueous ethanol as colourless plates, m. p. 137—139° (Found : C, 62·2; H, 8·6; N, 7·4; S, 8·2. Calc. for $C_{19}H_{32}O_3N_2S$: C, 62·0; H, 8·7; N, 7·6; S, 8·7%); OMe estimation of the acid gave erratic values (Found : OMe, 18·4, 12·8. Calc. for $C_{11}H_{22}O_3$: OMe, 15·35%). Fraction (ii), after crystallisation from acetone, had m. p. 76—77° (Found : C, 77·8; H, 12·8. Calc. for $C_{24}H_{48}O_2$: C, 78·2; H, 13·1%) (Ashton, Robinson, and Smith, *J.*, 1936, 283, give m. p. 84° for tetracosanoic acid). Its *amide*, after crystallisation from ethanol, had m. p. 112—113° (Found : C, 77·9; H, 13·3. $C_{24}H_{49}ON$ requires C, 78·4; H, 13·4%). The *p*-bromophenacyl ester, after crystallisation from ethanol, had m. p. 88—89° (Found : C, 67·5; H, 9·5. Calc. for $C_{32}H_{53}O_3Br$: C, 68·0; H, 9·4%) (Hann, Reid, and Jamieson, *J. Amer. Chem. Soc.*, 1930, **52**, 818, give m. p. 90—91° for the *p*-bromophenacyl ester of tetracosanoic acid). Examination of the infrared absorption of the acid and its amide (Nujol mulls) in the region 1350—1180 cm.$^{-1}$ (cf. Jones, McKay, and Sinclair, *J. Amer. Chem. Soc.*, 1952, **74**, 2575) indicated that the acid is tetracosanoic acid, and this was confirmed by the *X*-ray studies already described. The same acid was obtained by oxidation of phthiocerol with potassium permanganate in acetone, and also by drastic oxidation with chromic acid.

Mild Oxidation of Phthiocerol with Chromium Trioxide.—To a solution of phthiocerol (0·27 g., 0·0005 mole) in glacial acetic acid (150 c.c.) was slowly added a solution of chromium trioxide (0·066 g., 0·00066 mole) in glacial acetic acid (100 c.c.) at room temperature during 1 hr. The mixture was kept for 4 hr., then poured into water and extracted with ether. The extract was washed successively with aqueous sodium hydroxide and water, dried ($MgSO_4$), and evaporated. Ultraviolet light absorption of the crude product : max. 2200 and 2700 Å; ε 2700 and 2000, respectively. The infrared spectrum (film) indicated the presence of β-diketone (1610 and 1563 cm.$^{-1}$), free hydroxyl (3333 cm.$^{-1}$), and carboxyl groups (1706 cm.$^{-1}$). A solution of the product in ethanol–ether gave an immediate red colour on addition of anhydrous ferric chloride.

Conversion of Phthiocerol into a Dibromo-derivative.—Phthiocerol (2 g.) was kept with a solution of hydrogen bromide in glacial acetic acid (50% w/v; 25 c.c.) in a stoppered bottle at

the room temperature for 7 days. The mixture was then poured into water and extracted with ether. After being washed several times with water and dried ($MgSO_4$), the ethereal extract was evaporated, leaving a yellowish wax (Found : Br, 24·15. Calc. for $C_{36}H_{72}OBr_2$: Br, 23·5%).

Debromination of the Preceding Dibromide.—The dibromide (3·6 g.) was refluxed with dry acetone (100 c.c.), sodium iodide (10 g.), and zinc dust (6 g.) for 3 hr. The zinc dust was filtered off from the hot mixture and extracted with boiling chloroform (2 × 25 c.c.). The combined filtrates were poured into water and the whole was extracted with ether; the extract was washed with water, dried ($MgSO_4$), and evaporated. Distillation gave a bromine-free product, b. p. 255—265° (bath)/0·1 mm. (Found : C, 83·3; H, 13·8; no Br. Calc. for $C_{36}H_{72}O$: C, 83·1; H, 13·8%).

A portion (0·46 g.) of this product was refluxed with dry acetone (50 c.c.; previously distilled over $KMnO_4$) and potassium permanganate (0·5 g.) for 1 hr.; only 0·04 g. of acid was obtained; the bulk (0·38 g.) of the product appeared to be neutral.

Demethylation of Phthiocerol by the Action of Hydrogen Bromide and Debromination of the Product.—Phthiocerol (1·85 g.) was refluxed with a 50% w/v solution of hydrogen bromide in glacial acetic acid (20 c.c.) for 24 hr., and the mixture worked up as above. The resulting material (Found : Br, 26·9%), on debromination with zinc and sodium iodide in acetone by the procedure described in the preceding section, gave an unsaturated hydrocarbon (1·1 g.), b. p. 180° (bath)/0·5 mm. (Found : C, 86·1; H, 13·6. Calc. for $C_{35}H_{68}$: C, 86·1; H, 13·9%), showing in its infrared spectrum a band at 961·5 $cm.^{-1}$.

Ozonisation of the above Unsaturated Hydrocarbon.—Ozonised oxygen was passed through a solution of the hydrocarbon (1·1 g.) in dry carbon tetrachloride (25 c.c.) for 40 min. After removal of the solvent under reduced pressure at 40° (bath), water (25 c.c.) was added, and the mixture heated gradually to 100° (bath). During this operation volatile products were passed in a current of nitrogen into a receiver containing a solution of 2 : 4-dinitrophenylhydrazine in aqueous hydrochloric acid. A small quantity of dinitrophenylhydrazone was precipitated; this was dissolved in a little chloroform, and the solution examined (by Dr. H. S. Burton) by paper-chromatography according to the general procedure previously described (Burton, *Chem. and Ind.*, 1954, 576). On chromatograms including reference substances, run on Whatman No. 1 paper pretreated with diethyl tartrate (7·5% in aqueous methanol) or 70% aqueous ethyl lactate, and developed with light petroleum (b. p. 60—80°) containing 1·75% of carbon tetrachloride, spots corresponding to authentic specimens of the 2 : 4-dinitrophenylhydrazones of propaldehyde and methyl propyl ketone were detected.

The non-volatile material remaining in the residual aqueous mixture was isolated by ether-extraction, and the product refluxed with acetone (70 c.c.) and potassium permanganate (2 g.) for 1 hr. The precipitated manganese dioxide was filtered off, and the filtrate worked up as usual, to give a product, b. p. 220° (bath)/0·09 mm., which contained no acidic material and exhibited in its infrared spectrum a band at 1715 $cm.^{-1}$ (CO) (Found : C, 83·4; H, 13·4. Calc. for $C_{32}H_{62}O$: C, 83·1; H, 13·4%). The above manganese dioxide precipitate, after being worked up as usual, gave an acid of m. p. 69—71° (from acetone) which afforded an amide, b. p. 245° (bath)/0·06 mm. (Found : N, 3·6. Calc. for $C_{30}H_{59}ON$: N, 3·1%).

Reaction of Phthiocerol with Hydriodic Acid and De-iodination of the Product.—Phthiocerol (1 g.) was refluxed in a stream of nitrogen with constant-boiling hydriodic acid (10 c.c.; freshly distilled over red phosphorus) and phenol (1 g.) for 15 hr. (cf. Ginger and Anderson, *loc. cit.*). The mixture was cooled, poured into water, and distilled in steam to remove phenol. The residue was extracted with ether, and the ethereal solution washed with water, dried ($MgSO_4$), and evaporated. A pale-yellow wax (1·1 g.) was obtained which darkened in air (Found : I, 35·0. Calc. for $C_{35}H_{68}I_2$: I, 34·2; Calc. for $C_{35}H_{69}I_3$: I, 43·8%). This iodide (1 g.) was refluxed with acetone (80 c.c.) and zinc dust (5 g.) for 3 hr., and the mixture worked up as described above for a similar experiment involving the bromo-derivative. The product (0·42 g.) distilled at 200—220° (bath)/0·08 mm. (Found : C, 85·7; H, 14·0. Calc. for $C_{35}H_{70}$: C, 85·7; H, 14·3. Calc. for $C_{35}H_{68}$: C, 86·1; H, 13·9%). It showed a weak absorption band at 961·5 $cm.^{-1}$, and gave a low iodine value (15), indicating that a large proportion of the product had been reduced by the hydrogen iodide.

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