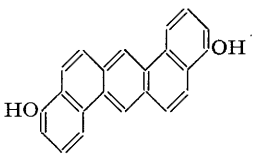
42. *Oxidation of Carcinogenic Hydrocarbons by Osmium Tetroxide.*

By J. W. Cook and (Miss) R. Schoental.

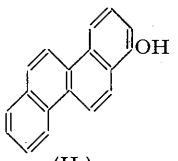
Ten polycyclic aromatic hydrocarbons, including some of the most potent cancer-producing compounds, have been oxidised by osmium tetroxide in benzene–pyridine to diols which undergo facile dehydration to hydroxy-derivatives of the original hydrocarbons. This process represents the closest parallel yet achieved to the biochemical oxidation of the hydrocarbons. The positions of attack are, however, different in the two processes. Thus, whereas metabolic oxidation of 1 : 2-benzanthracene leads to the 4′-hydroxy-derivative (III; R = H), chemical oxidation by the process now described leads to the 3-hydroxy-derivative (XI). Except in the case of chrysene the positions of the molecule which are attacked in metabolic oxidation and in oxidation with osmium tetroxide are both different from the positions which nearly all other chemical reagents show to be the most reactive positions.

In recent years considerable attention has been devoted to investigations of the metabolic transformations undergone in the animal body by polycyclic aromatic hydrocarbons, particularly those having cancer-producing activity. It has been supposed that these carcinogenic hydrocarbons, which on the whole are stable and rather inert, may exert their biological effects as a result of conversion into active metabolic products rather than by a direct action on the cells. The results of metabolic studies lend no support to this view, as the metabolic products, as far as they have been isolated, have proved to be either non-carcinogenic or less active than the hydrocarbons from which they are derived. The metabolic processes seem rather to represent a means of detoxification and elimination of the biologically active compounds.

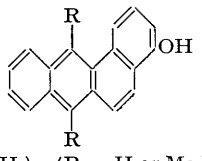
A common method of biochemical study has been to administer the hydrocarbon with the food of laboratory animals and to extract the excretion products from the urine or faeces, although other methods of administration have been used, and metabolic products have been detected in the tissues or body fluids. In many cases it has been found that elimination is preceded by hydroxylation, so that phenolic oxidation products are formed. Thus 1 : 2 : 5 : 6-dibenzanthracene is converted by rats and mice into the 4′ : 8′-dihdroxy-derivative (I) (Dobriner, Rhoads, and Lavin, *Cancer Research*, 1942, **2**, 95; Cason and Fieser, *J. Amer. Chem. Soc.*, 1940, **62**, 2681), and chrysene is converted into 3-hydroxychrysene (II) (Berenblum and Schoental, *Biochem. J.*, 1945, **39**, lxiv). There is strong evidence that the 4′-hydroxy-derivatives (III) are formed from 1 : 2-benzanthracene (Berenblum and Schoental, *Cancer Research*, 1943, **3**, 686) and 9 : 10-dimethyl-1 : 2-benzanthracene (Dickens and Weil-Malherbe, 22*nd Annual Report of the British Empire Cancer Campaign*, 1945, p. 55), and that 3 : 4-benzpyrene (IV) is converted into a mixture of its 8- and 10-hydroxy-derivatives (cf. Berenblum, Crowfoot, Holiday, and Schoental, *Cancer Research*, 1943, **3**, 151; Berenblum and Schoental, *ibid.*, 1946, **6**, 699).



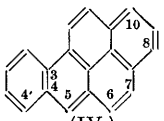
(I.)



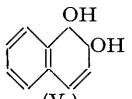
(II.)



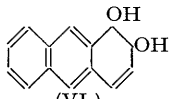
(III.) (R = H or Me.)



(IV.)



(V.)



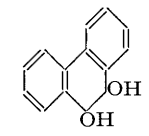
(VI.)

Oxidation of the hydrocarbon probably takes place through the intermediary of a diol formed by addition of hydroxyl groups to adjacent carbon atoms. Such diols (V and VI) have in fact been isolated from naphthalene (Young, *Canad. Chem.*, 1946, **30**, 124; *Biochem. J.*, 1947, **41**, 417) and anthracene (Boyland and Levi, *Biochem. J.*, 1935, **29**, 2679; Boyland and Shoppee, *J.*, 1947, 801). They are rapidly converted by treatment with dilute acid into α-naphthol and α-anthrol, respectively. Weigert and Mottram (*Cancer Research*, 1946, **6**, 109) have adduced spectroscopic evidence that there are intermediates in the biochemical oxidation of 3 : 4-benzpyrene to hydroxy-derivatives and have suggested that these intermediates are related to diols analogous to (V) and (VI).

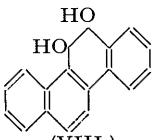
The positions in the molecules which are attacked in these biochemical oxidations are not those which have been revealed as the most reactive centres by purely chemical reagents. Thus, with 3 : 4-benzpyrene substitution almost invariably occurs in position 5, and this includes

oxidation by lead tetra-acetate which leads to the formation of the 5-acetoxy-derivative (Fieser and Hershberg, *J. Amer. Chem. Soc.*, 1938, **60**, 2542; 1939, **61**, 1565). Anthracene, 1 : 2-benzanthracene, and 1 : 2 : 5 : 6-dibenzanthracene are attacked at the reactive 9- and 10-positions of the anthracene system. This is reflected in substitution reactions, in oxidation to the quinones, in photo-oxidation to transannular peroxides, and in Diels–Alder addition of maleic anhydride and similar reagents. In general this is true also of homologues, although substitution may sometimes occur in reactive positions of side chains as in the lead tretra-acetate oxidation of 9 : 10-dimethyl-1 : 2-benzanthracene (cf. III) to the ωω′-diacetoxy-derivative (Badger and Cook, *J.*, 1939, 802). Eckhardt (*Ber.*, 1940 **73**, 13) compared the rates of oxidation of several polycyclic aromatic hydrocarbons by perbenzoic acid, but did not isolate the products.

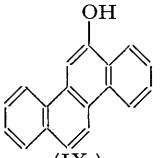
In view of these striking contrasts between the positions attacked in biochemical and in chemical oxidation it seemed of interest to examine the oxidation of polycyclic aromatic hydrocarbons by osmium tetroxide. An improvement on the original procedure of Criegee (*Annalen*, 1936, **522**, 75) was introduced by Criegee, Marchand, and Wannowius (*ibid.*, 1942, **550**, 99), who showed that if ethylenic compounds were treated with osmium tetroxide in presence of tertiary bases, especially pyridine, there were formed bright-coloured stable crystalline complexes having the character of esters of osmic acid. Such a complex was obtained by these authors from phenanthrene also, and underwent hydrolytic decomposition to 9 : 10-dihydroxy-9 : 10-dihydrophenanthrene (VII). We have found that this is a general reaction for polycyclic hydrocarbons containing a phenanthrene system. Chrysene was converted into 1 : 2-*dihydroxy*-1 : 2-*dihydrochrysene* (VIII) which was dehydrated by dilute acid to 2-chrysenol (IX); pyrene was converted into 1 : 2-*dihydroxy*-1 : 2-*dihydropyrene*, dehydrated to 1-pyrenol; and 1 : 2-benzanthracene was converted into 3 : 4-*dihydroxy*-3 : 4-*dihydro*-1 : 2-*benzanthracene* (X), dehydrated to 3-hydroxy-1 : 2-benzanthracene (XI). The last-named compound was identified by comparison of its methyl ether with synthetic 3-methoxy-1 : 2-benzanthracene prepared by the method of Fieser, Hershberg, Long, and Newman (*J. Amer. Chem. Soc.*, 1937, **59**, 475). Moreover, the diol (X) was oxidised by chromic acid to the known 1 : 2-benz-3 : 4-anthraquinone (Fieser and Dietz, *J. Amer. Chem. Soc.*, 1929, **51**, 3141).



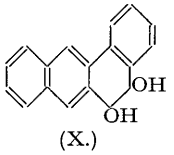
(VII.)



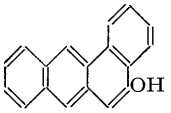
(VIII.)



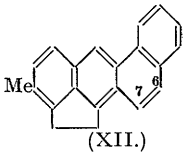
(IX.)



(X.)



(XI.)



(XII.)

Similar oxidation to *diols* by osmium tetroxide in benzene–pyridine followed by hydrolysis took place with 1′-methyl-, 9 : 10-dimethyl-, 5 : 9 : 10-trimethyl-, and 5 : 6 : 9 : 10-tetramethyl-1 : 2-benzanthracene, 1 : 2 : 5 : 6-dibenzanthracene, and 20-methylcholanthrene (XII). The diols were characterised by their *diacetates* and they were all readily dehydrated to phenolic hydroxy-compounds from which homogeneous *methyl ethers* were obtained by methylation. These diols and their dehydration products may be assumed by analogy to have structures corresponding with (X) and (XI), although the positions of the substituents have not been verified experimentally. It is reasonably certain, from the work of Criegee and his collaborators (*loc. cit.*) (cf. Böeseken, *Rec. Trav. chim.*, 1922, **41**, 199) that all the diols have the *cis*-configuration. It is of interest that catalytic hydrogenation of 20-methylcholanthrene (XII) leads in part to addition of hydrogen at the positions (6, 7) which are believed to be concerned in the addition of osmium tetroxide, although this is not so with 1 : 2-benzanthracene (Fieser and Hershberg, *J. Amer. Chem. Soc.*, 1937, **59**, 2502; 1938, **60**, 940).

3 : 4-Benzpyrene (IV) was also rapidly hydroxylated under the conditions used in these oxidations. The diol so formed was sensitive to atmospheric oxidation and could not be obtained completely pure, but it formed a well-crystallised *diacetate*. Dehydration by dilute acid gave a mixture of phenols from which, after methylation, two *methoxybenzpyrenes* were isolated. They were separated by fractional crystallisation of their picrates, combined with chromatographic purification. These two methoxybenzpyrenes differed from the known methoxybenz-

pyrenes (with substituents at positions 5, 10, and 4′) and were probably the 6- and 7-methoxy-compounds, the synthesis of which is being attempted.

The diols formed by hydroxylation of these polycyclic hydrocarbons by means of osmium tetroxide were obtained in good yield, and apart from the isolation of a small amount of 1 : 2 : 5 : 6-dibenz-3 : 4-anthraquinone as a by-product in the chromic acid oxidation of 1 : 2 : 5 : 6-dibenzanthracene (Cook, *J.*, 1933, 1592), their formation represents the first successful chemical oxidation of benzanthracene hydrocarbons in positions other than the reactive *meso*-positions of the anthracene system which they contain. The process obviously offers a very close parallel to the biochemical oxidation of the hydrocarbons, although the points of attack in the molecule are different. It may well be that the biochemical oxidation involves a similar formation of a metallic complex involving an oxidising enzyme system, but it remains a matter of conjecture why such a process should take place at some of the less reactive centres in the molecule. Possibly some form of steric influence is operative.

If osmium tetroxide oxidation could be effected in the simpler cases of naphthalene and anthracene, the metabolic diols (V and VI) could perhaps be obtained artificially. These hydrocarbons do indeed form coloured crystalline complexes with pyridine–osmium tetroxide, and the products which these yield on hydrolysis are being investigated.

Experimental.

Preparation of the Osmic Ester–Pyridine Complexes.—A solution (or suspension) of the hydrocarbon (0·0025 mole) in pure dry benzene (15 c.c.) was treated with osmium tetroxide (0·0025 mole) and purified pyridine (0·005 mole). The colour slowly deepened and the dark brown microcrystalline complex was deposited. With 3 : 4-benzpyrene and 1 : 2-benzanthracene and its homologues reaction was complete in 24 to 48 hours at room temperature, after which the decanted clear solution deposited no further precipitate. With less reactive or less soluble hydrocarbons (*e.g.*, anthracene, 1 : 2 : 5 : 6-dibenzanthracene) the reaction required several days or even weeks, at room temperature. With chrysene the reaction was completed in a week at 35°.

Formation of the Diols.—After complete separation from solution of the coloured complexes these were collected and dissolved in methylene chloride, and the solutions were shaken mechanically with 1% potassium hydroxide solution containing 10% of mannitol (cf. Criegee, Marchand, and Wannowius, *loc. cit.*). The colour of the methylene chloride solution faded and the aqueous solution became pink with potassium osmate. In some cases (pyrene, 3 : 4-benzpyrene, 1 : 2 : 5 : 6-dibenzanthracene) the methylene chloride solution remained somewhat coloured on account of quinone formation. Most of the diols were very sparingly soluble and partly came out of solution. The solid was filtered off and the remainder of the diol was recovered by evaporation of the methylene chloride solution. The diols, obtained in good yield, were recrystallised from benzene, chloroform, or methanol. It was found desirable to effect purification of some by chromatographic adsorption on alumina from benzene solution, followed by elution with chloroform. Most of the diols had poor power of crystallisation, and some of them tended to separate as gels from solutions in benzene or toluene. The diacetates, formed by brief boiling with acetic anhydride containing a little pyridine, were however well-crystallised colourless compounds. All the diols gave intense colours with concentrated sulphuric acid. The colours ranged from yellow or orange (pyrene, 3 : 4-benzpyrene) through scarlet or red (1 : 2-benzanthracene, 9 : 10-dimethyl-1 : 2-benzanthracene, 1 : 2 : 5 : 6-dibenzanthracene) to crimson (1′-methyl-, 5 : 9 : 10-trimethyl-1 : 2-benzanthracene) and intense purple (5 : 6 : 9 : 10-tetramethyl-1 : 2-benzanthracene). This last colour was especially persistent and lasted for several days. The diols from chrysene and 20-methylcholanthrene gave brown solutions in sulphuric acid.

3 : 4-*Dihydroxy*-3 : 4-*dihydro*-1 : 2-*benzanthracene* (X) formed colourless microscopic needles, m. p. 202—204° (decomp.) (Found: C, 82·6; H, 5·3. $C_{18}H_{14}O_2$ requires C, 82·45; H, 5·3%); its *diacetate* formed prisms, m. p. 142—143·5° (Found: C, 76·0; H, 5·1. $C_{22}H_{18}O_4$ requires C, 76·3; H, 5·2%). *Dihydroxy*-1′-*methyldihydro*-1 : 2-*benzanthracene* formed colourless silky needles (from benzene), m. p. 176—177° (Found: C, 82·8; H, 5·7. $C_{19}H_{16}O_2$ requires C, 82·6; H, 5·8%); its *diacetate* formed colourless rhombs, m. p. 145—146° (Found: C, 76·6; H, 5·6. $C_{23}H_{20}O_4$ requires C, 76·65; H, 5·6%). *Dihydroxy*-9 : 10-*dimethyldihydro*-1 : 2-*benzanthracene* formed long colourless needles (from methanol), m. p. 171—172° (Found: C, 82·95; H, 6·2. $C_{20}H_{18}O_2$ requires C, 82·75; H, 6·2%); its *diacetate* formed rhombs (from light petroleum), m. p. 153—153·5° (Found: C, 77·2; H, 5·7. $C_{24}H_{22}O_4$ requires C, 77·0; H, 5·9%). *Dihydroxy*-5 : 9 : 10-*trimethyldihydro*-1 : 2-*benzanthracene* formed colourless needles, m. p. 179—180° (Found: C, 82·6; H, 6·5. $C_{21}H_{20}O_2$ requires C, 82·9; H, 6·6%); a by-product, m. p. 187°, was formed in amount insufficient for complete purification. The *diacetate* from the pure diol formed thick rhombs (from light petroleum), m. p. 161—162° (Found: C, 77·5; H, 6·1. $C_{25}H_{24}O_4$ requires C, 77·3; H, 6·2%). The crude diol gave also by acetylation a compound which formed colourless leaflets, m. p. 209—210° (Found: C, 79·7; H, 6·0%). *Dihydroxy*-5 : 6 : 9 : 10-*tetramethyldihydro*-1 : 2-*benzanthracene* formed colourless needles (from light petroleum), m. p. 179—180° (Found: C, 83·3; H, 6·95. $C_{22}H_{22}O_2$ requires C, 83·0; H, 6·9%); its *diacetate* formed fine needles (from light petroleum), m. p. 156—157° (Found: C, 77·7; H, 6·3. $C_{26}H_{26}O_4$ requires C, 77·6; H, 6·5%). In this case the diol was obtained from the mother liquors of the crystallisation of the crude material from benzene. The compound which crystallised first from the benzene solution was a minor product which formed long colourless needles, m. p. 195—196° (Found: C, 78·5; H, 6·9%). *Dihydroxydihydro*-1 : 2 : 5 : 6-*dibenzanthracene* formed fine colourless needles (from toluene), m. p. *ca.* 200° (decomp.) (Found: C, 84·4; H, 5·0. $C_{22}H_{16}O_2$ requires C, 84·6; H, 5·1%); its *diacetate* was purified by chromatography on silica and formed colourless rhombs (from ethyl acetate), m. p. 176·5—177·5° (Found: C, 78·8; H, 5·1.

$C_{26}H_{20}O_4$ requires C, 78·75; H, 5·05%). *Dihydroxy-20-methyldihydrocholanthrene* formed a colourless solid (from benzene), m. p. *ca.* 215° (decomp.) (Found: C, 83·6; H, 5·8. $C_{21}H_{18}O_2$ requires C, 83·4; H, 6·0%); its *diacetate* formed needles, m. p. 210—211° (Found: C, 77·8; H, 5·8. $C_{25}H_{22}O_4$ requires C, 77·7; H, 5·7%). 1 : 2-*Dihydroxy*-1 : 2-*dihydrochrysene* (VIII), obtained by hydrolysis of the pure diacetate, formed an amorphous white solid (from benzene), m. p. *ca.* 180° (decomp.) (Found: C, 82·3; H, 5·4. $C_{18}H_{14}O_2$ requires C, 82·45; H, 5·3%); the *diacetate*, purified by chromatography on silica, formed needles (from light petroleum), m. p. 164—165° (Found: C, 76·5; H, 5·0. $C_{22}H_{18}O_4$ requires C, 76·3; H, 5·2%). 1 : 2-*Dihydroxy*-1 : 2-*dihydropyrene* formed an amorphous white solid (from benzene–light petroleum), m. p. *ca.* 178° (decomp.) (Found: C, 81·25; H, 5·25. $C_{16}H_{12}O_2$ requires C, 81·4; H, 5·1%) and gave a *diacetate* (purified by chromatography on silica and recrystallisation from light petroleum) as prisms, m. p. 146—147° (Found: C, 75·1; H, 4·9. $C_{20}H_{16}O_4$ requires C, 75·0; H, 5·0%). The *diol* from 3 : 4-benzpyrene was very sensitive to atmospheric oxidation and all attempts at purification gave a pink amorphous product, m. p. 200—215° (decomp.) (Found: C, 83·5; H, 4·7. $C_{20}H_{14}O_2$ requires C, 83·9; H, 4·9%); its *diacetate* formed colourless prisms (from benzene), m. p. 214—215° (Found: C, 78·0; H, 4·9. $C_{24}H_{18}O_4$ requires C, 77·8; H, 4·9%).

Dehydration of the Diols and Formation of Methoxy-derivatives of the Hydrocarbons.—Dehydration was readily effected by dilute mineral acid. For example, a solution of the diol (0·1 g.) in acetic acid (3 c.c.) containing a few drops of concentrated hydrochloric acid was boiled for a few minutes, cooled, and diluted with water to precipitate the phenol. Most of the phenols were sensitive to atmospheric oxidation, so that the recrystallised products were contaminated with quinones. They were therefore characterised as their methyl ethers, prepared by methylation of the crude phenols with methyl sulphate in alkaline solution. These methyl ethers were usually purified by chromatography on alumina, followed by recrystallisation from light petroleum.

3-Methoxy-1 : 2-benzanthracene (cf. XI) formed colourless needles, m. p. 166—167°, not depressed by admixture with an authentic sample synthesised by the method of Fieser *et al.* (*loc. cit.*). Acetylation of the crude dehydration product of (X) gave 3-acetoxy-1 : 2-benzanthracene, m. p. 126—127° (Fieser and Dietz, *loc. cit.*, give m. p. 129°). In the chromatographic purification of this acetate there was isolated the 1 : 2-benz-3 : 4-anthraquinone of Fieser and Dietz, which was also obtained by chromic acid oxidation of the diol (X) in acetic acid.

3(?)-*Methoxy*-1′-*methyl*-1 : 2-*benzanthracene* formed colourless needles, m. p. 132—133° (Found: C, 88·2; H, 5·6. $C_{20}H_{16}O$ requires C, 88·2; H, 5·9%); 3(?)-*benzoxy*-1′-*methyl*-1 : 2-*benzanthracene* formed almost colourless fine needles, m. p. 192—193° (Found: C, 85·8; H, 5·05. $C_{26}H_{18}O_2$ requires C, 86·2; H, 5·0%). 3(?)-*Methoxy*-9 : 10-*dimethyl*-1 : 2-*benzanthracene* formed almost colourless rhombic plates, m. p. 129—130° (Found: C, 88·2; H, 6·2. $C_{21}H_{18}O$ requires C, 88·1; H, 6·3%); 3(?)-*methoxy*-5 : 9 : 10-*trimethyl*-1 : 2-*benzanthracene* formed pale yellow lances, m. p. 145—146° (Found: C, 87·8; H, 6·6; OMe, 10·0. $C_{22}H_{20}O$ requires C, 88·0; H, 6·7; OMe, 10·3%); 3(?)-*methoxy*-5 : 6 : 9 : 10-*tetramethyl*-1 : 2-*benzanthracene* formed long yellow needles, m. p. 164·5—165° (Found: C, 87·6; H, 6·9; OMe, 10·65. $C_{23}H_{22}O$ requires C, 87·9; H, 7·0; OMe, 10·4%). 3(?)-*Methoxy*-1 : 2 : 5 : 6-*dibenzanthracene* was purified through its crimson picrate, m. p. 183° (decomp.), and then formed fine colourless needles, m. p. 200—201° (Found: C, 89·5; H, 5·0. $C_{23}H_{16}O$ requires C, 89·6; H, 5·2%). 6(?)-*Methoxy*-20-*methylcholanthrene* (cf. XII) formed pale yellow needles, m. p. 204—205° (Found: C, 88·5; H, 6·3. $C_{22}H_{18}O$ requires C, 88·6; H, 6·0%). 2-Methoxychrysene (from IX) had m. p. 125—126°, not depressed by a specimen prepared through the 2-sulphonic acid (Newman and Cathcart, *J. Org. Chem.*, 1940, **5**, 621). 1-*Methoxypyrene* formed thick colourless prisms, m. p. 128—129° (Found: C, 87·8; H, 5·1; OMe, 13·9. $C_{17}H_{12}O$ requires C, 87·9; H, 5·2; OMe, 13·4%). This m. p. differed from those of the known 3- and 4-methoxypyrenes. For confirmation that the new compound, obtained through the diol, was the hitherto unknown 1-methoxypyrene, a specimen of this was prepared by a method which established its structure. For this purpose, *s*-hexahydropyrene (Cook and Hewett, *J.*, 1933, 398) was sulphonated with concentrated sulphuric acid at 70° (compare Schroeter, *Ber.*, 1924, **57**, 2022). The sodium sulphonate was fused with potash at 280—290°, the crude phenol was methylated with methyl sulphate and alkali, and the liquid product was dehydrogenated by heating with palladium-black at 270°. Chromatographic purification followed by recrystallisation then gave 1-methoxypyrene, m. p. 128—129° alone or mixed with the compound prepared from the diol formed by osmium tetroxide oxidation of pyrene.

Treatment of the diol from 3 : 4-benzpyrene gave a mixture of methoxybenzpyrenes. This was the only case in which there was any evidence of formation of more than one methoxy-compound by dehydration of a diol followed by methylation. The dihydroxydihydrobenzpyrene obtained by hydrolysis of its pure diacetate gave the same mixture of methoxybenzpyrenes as was formed from the crude diol. Partial separation of the mixture was effected by chromatography on alumina using light petroleum as solvent (coloured zones which appeared to contain quinones were also formed). The separated methoxybenzpyrenes were then converted into picrates, which were fractionally crystallised from benzene. In this way were obtained (*a*) brown needles, m. p. 173—174°, which were freed from picric acid to give a *methoxy*-3 : 4-*benzpyrene*, flat yellow needles (from light petroleum), m. p. 136—137° (Found: C, 89·5; H, 5·1. $C_{21}H_{14}O$ requires C, 89·4; H, 5·0; OMe, 11·0%); (*b*) brown needles, m. p. 178—179°, which yielded an isomeric *methoxy*-3 : 4-*benzpyrene* as small yellow needles (from light petroleum), m. p. 179·5—180° (Found: C, 89·3; H, 4·95; OMe, 11·4%). This isomeride was more strongly adsorbed on alumina than the compound, m. p. 136°, and its m. p. was strongly depressed by admixture with 4′-methoxy-3 : 4-benzpyrene, m. p. 183—184° (Fieser, Hershberg, and Newman, *J. Amer. Chem. Soc.*, 1935, **57**, 1509).

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