changed I (peak A) was identified by recrystallization with carrier from water to constant specific activity. The radioactivity in peak B could be volatilized by treatment with ammonia and acidification with formic acid, identifying it indirectly as II.[7] Peak D was shown to be hydantoin-5-propionic acid (IV) by recrystallization with carrier to constant specific activity from both ethanol–benzene and water and co-chromatography in six solvents with the synthetic compound.[8] Synthetic IV (Found: C, 41.69; H, 4.60; N, 16.29) prepared from L-glutamic acid[9] was eluted in the identical position as peak D. The dotted line in Fig. 1 is synthetic radioactive IV. Peaks A–D have 72, 1.1, 1.5 and 8.5% of the urinary radioactivity, respectively.

Incubation of I with rat liver slices forms small amounts of IV in the presence or absence of III; radioactive IV is present in monkey and human urine after intravenous C^{14} histidine.

The biochemical steps from I to IV have not yet been elucidated.

Acknowledgment.—The authors are grateful to Dr. Herbert Tabor for his helpful criticism.

(7) B. A. Borek and H. Waelsch, *J. Biol. Chem.*, **205**, 459 (1953).

(8) Synthetic IV sprayed with 0.1 M AgNO$_3$: 0.1 M NH$_4$OH (1:1) is white against a brown background. Radioautograph spots matched the outline and position of stained spots exactly. R_F values for benzene: 1-butanol:methanol:H$_2$O (1:1:2:1), 2-butanol:formic acid:H$_2$O (19:2:6), acetic acid:1-butanol:ethyl acetate:H$_2$O (1:1:1:1), ethanol:ether:H$_2$O:7.4 N NH$_3$ (4:5:1:0.1), 1-propanol:1 N acetic acid (3:1), 2-propanol:NH$_3$:H$_2$O (8:1:1) were 0.64, 0.67, 0.68, 0.13, 0.69, 0.07, respectively.

(9) H. D. Dakin, *Biochem. J.*, **13**, 398 (1919).

LABORATORY OF CLINICAL SCIENCE
NATIONAL INSTITUTE OF MENTAL HEALTH
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND

DONALD D. BROWN
MARIAN W. KIES

RECEIVED JULY 31, 1958

MICROBIOLOGICAL TRANSFORMATIONS. III.[1] THE HYDROXYLATION OF STEROIDS AT C-9

Sir:

We wish to report the microbiological preparation and proof of structure of 9α-hydroxy-4-androstene-3,17-dione. This compound and the method used to establish its structure may help resolve the difficulties previously experienced in the formulation of 8- or 9-hydroxysteroids.[2]

Fermentation of 4-androstene-3,17-dione, by the methods previously described,[3] with a species of Nocardia (A20-10) isolated from soil produced 9,10-seco-3-hydroxy-1,3,5(10)-androstatriene-9,17-dione[1] and a monohydroxy-4-androstene-3,17-dione (I), m.p. 222–223.5°; $\lambda_{\text{max.}}^{\text{methanol}}$ 241 mμ (ϵ 16,100); $[\alpha]_D$ +181.7° (CHCl$_3$); $\lambda_{\text{max}}^{\text{KBr}}$ 2.92 μ (—OH), 5.76 μ (17 C═O), 6.02 μ and 6.19 μ (3 C═O, Δ^4); (found: C, 75.21; H, 8.68). The hydroxy-4-androstene-3,17-dione (I) was recovered unchanged when treated with pyridine and acetic anhydride. Fermentation of I with a species of Arthobacter (B 20-178) that converts 4-androstene-3,17-dione to 1,4-androstadiene-3,17-dione in excellent yield, gave 9,10-seco-3-hydroxy-1,3,5(10)-androstatriene-9,17-dione. The latter compound was purified as its acetate, m.p. 143.5–146°, which proved to be identical in all respects (m.p., mixed m.p., and infrared) with the 9,10-seco-3-acetoxy-1,3,5(10)-androstatriene-9,17-dione reported previously.[1] Thus, the positions of the three oxygen atoms in I were established. The 9α-configuration was assigned to the new hydroxyl group because of its molecular rotatory contribution ($\Delta M_D^{\text{OH—H}} = -18$)[4] and because of the recent evidence that microbiologically introduced hydroxyl groups have the same configuration as the hydrogens replaced.[5]

In the aromatization-degradation of 4-androstene-3,17-dione it seems probable that this species of Nocardia[6] first hydroxylates at C-9 then introduces the Δ^1-double bond. This is just the opposite sequence originally found with *Pseudomonas*.[1] A paper chromatographic study of the fermentation of 9α-hydroxy-4-androstene-3,17-dione with *Pseudomonas* showed the formation of, at most, only trace quantities of phenolic material. With Pseudomonas the sequence in which the reactions occur seems to be limited.

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(1) Previous paper: R. M. Dodson and R. D. Muir, THIS JOURNAL, **80**, 5004 (1958). The numbers assigned to the organisms are our laboratory designations.

(2) (a) S. H. Eppstein, P. D. Meister, D. H. Peterson, H. C. Murray, H. M. Leigh Osborn, A. Weintraub, L. M. Reineke and R. C. Meeks, *ibid.*, **80**, 3382 (1958); (b) D. Stone, M. Hayano, R. I. Dorfman, O. Hechter, C. R. Robinson and C. Djerassi, *ibid.*, **77**, 3926 (1955).

(3) D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, P. D. Meister and H. M. Leigh, *ibid.*, **74**, 5933 (1952).

(4) The molecular rotatory contribution of the 9α-hydroxyl group in 3β-acetoxyergostan-9α-ol was −31. A. S. Hallsworth and H. B. Henbest, *J. Chem. Soc.*, 4604 (1957). The molecular rotatory contribution of the new (8 or 9) hydroxyl group in the steroids hydroxylated with *Helicostylum piriforme*, *Mucor parasiticus*, *Mucor griseocyanus* and *Neurospora crassa* (Ref. 2) indicates the probability of 9α, rather than 8β, hydroxylation. See: S. H. Eppstein, P. D. Meister, H. C. Murray and D. H. Peterson, "Vitamins and Hormones," Vol. XIV, 388 (1956), Academic Press, Inc., New York, N. Y. However, the specific rotation of the previously described 8β (or 9α)-hydroxy-4-androstene-3,17-dione,[2a] m.p. 214–217°, $[\alpha]_D$ +165° (CHCl$_3$), obtained via the hydroxylation of 11-deoxycortisol with *H. Piriforme*, does not agree with ours.

(5) (a) M. Hayano, M. Gut, R. I. Dorfman, O. K. Sebek and D. H. Peterson, THIS JOURNAL, **80**, 2336 (1958); (b) E. J. Corey, G. A. Gregoriou and D. H. Peterson, *ibid.*, **80**, 2338 (1958).

(6) We have isolated another strain of Nocardia (A20-9) which apparently follows the alternate sequence.

G. D. SEARLE AND COMPANY
P. O. BOX 5110
CHICAGO 80, ILLINOIS

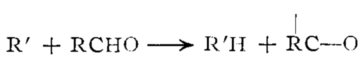
R. M. DODSON
R. D. MUIR

RECEIVED SEPTEMBER 24, 1958

FREE RADICAL ADDITION OF CYCLOPENTANE AND CYCLOHEXANE TO FORMALDEHYDE

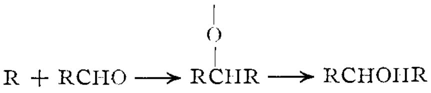
Sir:

Although alkyl free radical attack upon an aldehyde is most likely to result in hydrogen abstraction[1]



$$\text{R}' + \text{RCHO} \longrightarrow \text{R}'\text{H} + \text{R}\overset{|}{\text{C}}\text{—O} \qquad (1)$$

addition to the carbonyl group also has been demonstrated.[2]



$$\text{R} + \text{RCHO} \longrightarrow \text{R}\overset{\overset{|}{\text{O}}}{\overset{|}{\text{C}}}\text{HR} \longrightarrow \text{RCHOHR} \qquad (2)$$

Consideration of reaction (2) leads to the conclusion that it should be possible to bring about

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(1) For summary, see Steacie, "Atomic and Free Radical Reactions," 2nd Ed., Reinhold Publishing Corp., New York, N. Y., 1954.

(2) F. F. Rust, F. H. Seubold and W. E. Vaughan, THIS JOURNAL, **70**, 4253 (1948).

a free radical reaction between a saturated hydrocarbon and formaldehyde. For example, if di-*tert*-butyl peroxide were to be the initiator the steps would be

$$\begin{gathered}(CH_3)_3COOC(CH_3)_3 \longrightarrow 2(CH_3)_3CO\text{—} \\ (CH_3)_3CO\text{—} + RH \longrightarrow (CH_3)_3COH + R \\ R + HCHO \longrightarrow RCH_2O\text{—} \\ RCH_2O + RH \longrightarrow RCH_2OH + R\end{gathered}$$

This sequence of reactions does in fact take place between cyclohexane or cyclopentane and formaldehyde with formation of cycloalkyl carbinols.

The reaction was carried out in a Teflon-sealed glass polymerization bottle which was shaken for 12 hours at 135°. A two phase reaction mixture of one mole cyclohexane, 0.25 mole of formaldehyde as a 37% formalin solution, and 0.1 mole of di-*tert*-butyl peroxide gave a 38% yield (based on formaldehyde) of cyclohexyl carbinol. *Anal.* Calcd. for $C_7H_{14}O$: C, 73.6; H, 12.3. Found: C, 73.5; H, 12.3. The phenylurethan derivative melted at 82.5–83° and showed no depression when mixed with a phenylurethan derivative of authentic cyclohexyl carbinol. Comparison of the infrared spectrum of this material with that of known cyclohexyl carbinol confirmed the identity of the product.

Similarly, five moles of cyclopentane, one mole of formaldehyde and 0.05 mole of di-*tert*-butyl peroxide yielded 21.4% of cyclopentyl carbinol. Separation of the product from a small amount of unknown contaminant required gas–liquid chromatography. *Anal.* Calcd. for $C_6H_{12}O$: C, 72.0; H, 12.1. Found: C, 71.8; H, 12.0. A phenylurethan derivative of this carbinol melted at 108° (lit. value 108°)[3]; *anal.* Calcd. for $C_{13}H_{17}NO_2$: C, 71.2; H, 7.8. Found: C, 71.3; H, 7.8.

[3] S. Nametkin and O. Morosowa, *Chem. Zentr.*, **87**, II, 250 (1916).

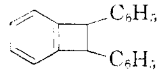
SHELL DEVELOPMENT COMPANY GLENN FULLER
EMERYVILLE, CALIFORNIA FREDERICK F. RUST

RECEIVED AUGUST 11, 1958

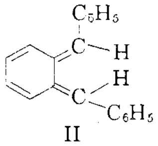
UNSATURATED FOUR-MEMBERED RING COMPOUNDS. II. 1,2-DIPHENYLBENZOCYCLOBUTENE, A COMPOUND HAVING UNUSUAL REACTIVITY

Sir:

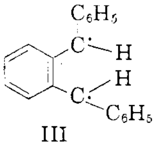
The compound 1,2-diphenylbenzocyclobutene (I) has been prepared by the reaction of sodium iodide with α,α'-dibromo-α,α'-diphenyl-*o*-xylene and by the reaction of bases with α-bromo-α,α'-diphenyl-*o*-xylene. White needles from alcohol, m.p. 94.5–95.2° (in absence of strong light), *Anal.* Calcd. for $C_{20}H_{16}$: C, 93.72; H, 6.28; M.W., 256. Found: C, 93.71; H, 6.27; M.W., 280.



I



II



III

Oxidation with chromic anhydride converts I to *o*-dibenzoylbenzene. The compound I is obtained essentially unchanged from hot alcohol and other solvents and does not decompose upon standing. The infrared spectrum has a band at 10.0 μ which is characteristic of a cycloalkane ring[1] and is common to a variety of compounds containing the benzocyclobutene ring system.[2,3] The observed values of the extinction coefficients in the ultraviolet spectrum are in agreement with the sum of the extinction coefficients of two toluene molecules and a benzocyclobutene molecule: $\lambda_{\max}^{\mathrm{MeOH}}$ 260 mμ (log ϵ = 3.24), 266 mμ (log ϵ = 3.36), 272 mμ (log ϵ = 3.32). Thus, the evidence strongly indicates that the major species present in solution is I and not II or III.

The main interest concerning I is its high degree of reactivity toward a number of reagents, a few examples of which are given here. By contrast, benzocyclobutene is inert in all of these reactions under comparable conditions.[4] With a molar amount of maleic anhydride in carbon tetrachloride at room temperature crystals appear after about two hours and after forty-eight hours a nearly quantitative yield of analytically pure 1,4-diphenyl-1,2,3,4-tetrahydronaphthalene-1,2-dicarboxylic anhydride is obtained with m.p. 219.2–219.8°. *Anal.* Calcd. for $C_{24}H_{18}O_3$: C, 81.33; H, 5.12; mol. wt., 354. Found: C, 81.33; H, 5.25; mol. wt., 335. Treatment of (I) with sulfur dioxide for fifteen minutes in refluxing carbon tetrachloride or for twenty-three hours at room temperature gives a nearly quantitative yield of the analytically pure 1,3-diphenyl-1,3-dihydroisothianaphthene-2,2-dioxide. *Anal.* Calcd. for $C_{20}H_{16}SO_2$: C, 74.97; H, 5.03; S, 10.0; mol. wt., 320. Found: C, 74.81; H, 5.12; S, 9.8; mol. wt., 310. This sulfone decomposes at 232.5–234° with evolution of sulfur dioxide to yield chiefly 9-phenyl-9,10-dihydroanthracene.[5] Compound I is readily reduced by hydrogen in alcohol at room temperature with palladium on charcoal to yield *o*-dibenzylbenzene.

The simplest explanation for these observations would seem to be that II or III is formed from I in a small equilibrium amount even at room temperature. However, the low degree of reactivity of a solution of I with alcohol, water, bases and oxygen is not consistent with the high degree of reactivity expected of compounds possessing the *o*-quinoid type structure or of radicals, although structure II might be expected to possess a stability greater than that of many compounds possessing the *o*-quinoid structure. The possibility is also being considered that the reagents react directly with I. For example, the reaction with maleic anhydride could reasonably occur by a four-center type mechanism. Further work is being carried out in order to decide between these possibilities.

DEPARTMENT OF CHEMISTRY FREDERICK R. JENSEN
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIF. WILLIAM E. COLEMAN

RECEIVED SEPTEMBER 2, 1958

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[1] L. W. Marrison, *J. Chem. Soc.*, 1614 (1951).

[2] M. P. Cava and D. R. Napier, THIS JOURNAL, **78**, 500 (1956).

[3] Unpublished data.

[4] Benzocyclobutene reacts analogously but only at elevated temperatures.

[5] Recently it was reported that 1,3-dihydroisothianaphthene-2,2-dioxide decomposes at temperatures above 280° to form benzocyclobutene and other products. Adducts are formed when the reactions are carried out in the presence of dienophiles. M. P. Cava and A. Deana, Abstracts of the 134th Meeting of the American Chemical Society, page 7P, Chicago, Illinois, September, 1958.