known to become radioactive under the same experimental conditions when C^{14}-acetate is the carbon source.[7] If the side-chain substitution occurred at the squalene stage, a radioactive hydrocarbon containing 31 carbon atoms and presumably having the same chromatographic properties as squalene would be an expected intermediate. Since formate failed to contribute any radioactivity to the hydrocarbon fraction, it is likely that the one-carbon unit is attached to an already completed steroid structure.

An inoculum of Fleischmann's baker's yeast was grown anaerobically for 48 hours.[8] The cells were harvested by centrifugation (yield 4.8 g., wet weight) and were suspended in 20 vol. of 0.1 *M* KH_2PO_4 containing 1% glucose, and aerated for 1.5 hours. The cells were again centrifuged, resuspended in 30 ml. of 0.03 *M* phosphate buffer containing 1% glucose and 2 mg. of C^{14} formate (60 μc.) and incubated in air at 25° for 24 hours. The non-saponifiable fraction extracted from the cells contained 7.4×10^5 c.p.m. (1.2% of the C^{14} added). Chromatography on deactivated (7% acetic acid) alumina yielded no significant radioactivity in the petroleum ether eluate (hydrocarbon fraction) and on elution with 14% benzene in petroleum ether, 6.6×10^5 c.p.m. in the ergosterol fraction. Carrier ergosterol was added and the mixture crystallized three times from methanol–chloroform to yield material having 29 c.p.m./mg. This ergosterol was degraded according to Hanahan and Wakil[9] with the modification that the methyl isopropyl ketone was isolated as the semicarbazone before degradation to iodoform.

TABLE I

INCORPORATION OF C^{14}-FORMATE INTO ERGOSTEROL AND DEGRADATION PRODUCTS

	C.p.m./m.g.[a] Found	Calcd.[c]	C.p.m.[b] Found	Calcd.[c]
Ergosterol	29	..	15	...
DNP-α,β-dimethyl-butyraldehyde[d]	40	41	34	35
Methyl isopropyl ketone semicarbazone[e]	78	80	65	70
Iodoform (C_{28})	..	..	310[f]	420

[a] Infinitely thin samples. [b] Infinitely thick samples of $BaCO_3$. [c] Calcd. on the assumption that C_{28} is the only labeled carbon atom. [d] M.p. 123–125°, reported[3] 124–125°. [e] M.p. 112–114°, reported 112°.[9] [f] The liquid combustion of iodoform has been reported[10] to give $BaCO_3$ of lower specific activity than expected. The low value (74% of calcd.) in the iodoform reported here may therefore be ascribed, at least in part, to this analytical difficulty.

Acknowledgment.—This work was supported by a grant-in-aid from Eli Lilly and Company.

(7) J. D. Johnston and K. Bloch, THIS JOURNAL, in press.
(8) H. P. Klein, *J. Bacteriology*, **69**, 620 (1955).
(9) G. Ciamician and P. Silber, *Ber.*, **43**, 947 (1910).
(10) G. Ehrensvärd, Q. Reio, E. Saluste and R. Stjernholm, *J. Biol. Chem.*, **189**, 93 (1951).

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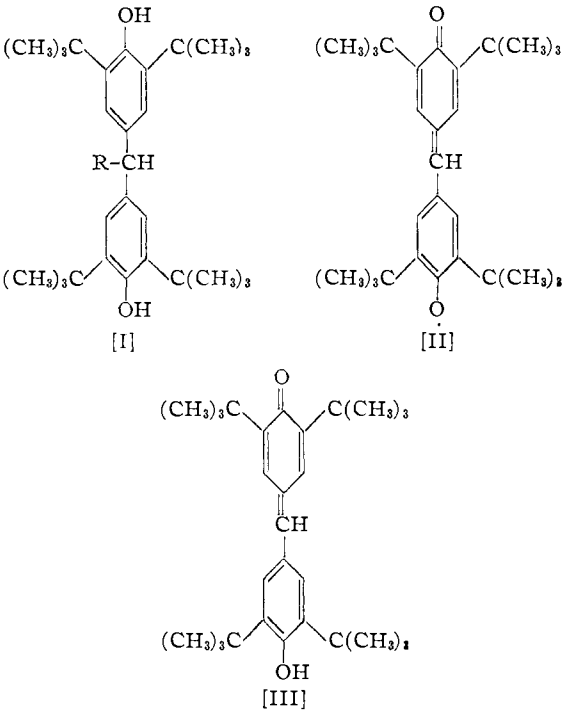
RECEIVED DECEMBER 17, 1956

A STABLE PHENOXY RADICAL INERT TO OXYGEN

Sir:

A very dark blue crystalline compound [II] has been isolated from oxidation of 3,3′,5,5′-tetra-*tert*-butyl-4,4′-dihydroxydiphenylmethane [I] R is H, by lead dioxide in ether or isoöctane



Compound II has m.p. 153°. *Anal.* Calcd. for $C_{29}H_{41}O_2$: C, 82.6; H, 9.7. Found: C, 82.8, 82.7; H, 9.9, 9.9. This corresponds to the loss of three hydrogens from the starting phenol.

Compound II is a radical. Magnetic susceptibility measurements indicate that there is one unpaired electron per molecule in the solid state. The electron magnetic resonance spectrum of II is a singlet both in the solid state and in isoöctane solution, "*g*" value of 2. The unique property of II is its unreactivity toward oxygen. No reaction occurs in the solid state after three months and none in isoöctane after three days. The radical character decays slowly in solution but not through reaction with oxygen.

The infrared spectrum indicates no OH stretching and the only absorption between 3.5 and 6.4μ is an intense band at 6.35μ. This band has been reported by Cook and by Mueller to be present in the spectra of phenoxy radicals derived from oxidation of hindered phenols.[1,2]

The ultraviolet spectrum indicates that over a concentration range variation of twenty-five Beer's law is obeyed; measurements were made on a very strong band at 420 mμ, ϵ_{molar} 200,000.

Compound II is reduced to III with hydroquinone in ether; it is reduced to the parent phenol or to compound III by hydrogen and platinum. Compound III (m.p. 157–158°, *Anal.* Calcd. for

(1) C. C. Cook and B. E. Norcross, THIS JOURNAL, **78**, 3797 (1956).
(2) I. E. Mueller, K. Ley and W. Kiedaisch, *Ber.*, **88**, 1819 (1955).

$C_{29}H_{42}O_2$: C, 82.6; H, 10.0. Found: C, 82.4; H, 10.0) is oxidized to II by lead dioxide in ether. Compound III has an OH stretching band in the infrared at 2.74 μ and three intense bands between 6.1 and 6.5 μ. Methanol adds to the quinone methide to yield the bisphenol I, R is OCH_3, m.p. 160–161°. *Anal.* Calcd. for $C_{30}H_{46}O_3$: C, 79.5; H, 10.2. Found: C, 79.3; H, 10.0, 10.3.

Compound II is sensitive to traces of strong acid in hydroxylic or hydrocarbon solvents. The radical is converted in methanol with a trace of toluenesulfonic acid to I, R is OCH_3 and 3,3′,5,5′-tetra-*tert*-butyl-4,4′-diphenoquinone, m.p. 239–240°. Base transforms II to a different radical which reacts rapidly with oxygen. These reactions will be described later.

Compound II is being investigated as a standard for electron magnetic resonance measurements.

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RECEIVED DECEMBER 6, 1956

SOME NEW ACTIVE CORTICOSTEROIDS

Sir:

The enhancement of the glucocorticoid[1] and antiarthritic[2] activities of prednisone (I)[3] and prednisolone (II)[4] relative to cortisone (III) and cortisol (IV), led us to introduce further unsaturation into cortical hormones. To this end, we have prepared 6-dehydroprednisone (V) and 6-dehydroprednisolone (VI).

Bromination of the 21-acetate of I with N-bromosuccinimide[5] gave 6-bromo-1,4-pregnadiene-17α,21-diol-3,11,20-trione 21-acetate (VII) (m.p. 185–188° (dec.); $[\alpha]^{25}D$ +173° (dioxane); λ_{max}^{EtOH} 245 mμ (ϵ = 16,300); found: Br, 16.54). Dehydrobromination of VII in refluxing collidine gave 1,4,6-pregnatriene-17α,21-diol-3,11,20-trione, (V) 21-acetate (m.p. 225–228°; $[\alpha]^{25}D$ +265° (dioxane); λ_{max}^{EtOH} 222 mμ (11,400), 255 (10,300), 297 (12,100); λ_{max}^{Nujol} 2.96 μ (OH), 5.76 (20 C=O, 21-OAc), 5.85 (11 C=O), 6.02 (3 C=O), 6.21, 6.31 ($\Delta^{1,4,6}$); found: C, 69.18; H, 6.73). The 21-acetate of V also was obtained by dibromination of the 21-acetate of III in acetic acid, followed by dehydrobromination with collidine.

An alternate procedure was the microbiological dehydrogenation of 6-dehydrocortisone (VIII)[6] or its 21-acetate with *Bacillus sphaericus*,[6] leading directly to V, two forms (m.p. 235° (dec.); 225° (dec.); $[\alpha]^{25}D$ +246° (dioxane); λ_{max}^{MeOH} 222 μ (11,100), 255 (9,900), 296 (11,700); $\lambda_{max}^{CHBr_3}$ 2.79, 2.88 μ (OH), 5.85 (11, 20 C=O), 6.06 (3 C=O), 6.13, 6.24, 6.31 ($\Delta^{1,4,6}$); found: C, 70.83; H, 6.81).

6-Dehydrocortisol (IX) (m.p. 239–241°; $[\alpha]^{25}D$ +177° (dioxane); λ_{max}^{MeOH} 283 mμ (24,900); λ_{max}^{Nujol} 2.97 μ (OH), 5.84 (20 C=O), 6.11 (3 C=O), 6.20, 6.33 ($\Delta^{4,6}$); found: C, 69.86; H, 8.06) was prepared from the 3-semicarbazone 21-acetate of VIII[6] by formation of the 3,20-bis-semicarbazone 21-acetate of VIII[7] (darkens 250°, m.p. >320°; λ_{max}^{MeOH} 242 mμ (14,500), 301 (40,200); λ_{max}^{Nujol} 2.89, 2.98, 3.05 μ (OH, NH), 5.79 (ester C=O), 5.86 (11 C=O), 5.99 (amide C=O), 6.35 ($\Delta^{4,6}$), 7.99 (ester C–O–C); found: N, 16.46), reduction with sodium borohydride to the 3,20-bis-semicarbazone of IX (darkens 260°, m.p. > 320°; λ_{max} 236 mμ (11,100), 298 (37.400); λ_{max}^{Nujol} 2.93, 3.06 μ (OH, NH), 5.99 (amide C=O), 6.40 ($\Delta^{4,6}$); found: N, 16.27), and cleavage with pyruvic acid and *p*-toluenesulfonic acid. Microbiological dehydrogenation of IX with *B. sphaericus* gave 1,4,6-pregnatriene-11β,17α,21-triol-3,20-dione (VI) (m.p. 239–243°; $[\alpha]^{25}D$ +100° (dioxane); λ_{max}^{MeOH} 222 mμ (11,600), 256 (9,600), 298 (12,200); λ_{max}^{Nujol} 2.85, 2.93, 3.00μ (OH), 5.84 (20 C=O), 6.09 (3 C=O), 6.20, 6.27, 6.34 ($\Delta^{1,4,6}$); found: C, 70.25; H, 7.26).

The structure is supported by the preparation of 1,4,6-androstatriene-3,11,17-trione (X) (m.p. 215° (dec.); $[\alpha]^{25}D$ +309° (acetone); λ_{max}^{MeOH} 222 mμ (11,300), 255 (9,900), 296 (11,900); λ_{max}^{Nujol} 5.73 μ (17 C=O), 5.84 (11 C=O), 6.06 (3 C=O), 6.23, 6.34 ($\Delta^{1,4,6}$); found: C, 77.00; H, 6.67), both from degradation of V with sodium bismuthate[1b,8] and from 1,4-androstadiene-3,11,20-trione[1b] by reaction with N-bromosuccinimide[5] to give 6-bromo-1,4-androstadiene-3,11-20-trione (m.p. 168° (dec.); $[\alpha]^{25}D$ +197° (dioxane); λ_{max}^{MeOH} 243 mμ (15,300); λ_{max}^{Nujol} 5.74 μ (17 C=O), 5.85 (11 C=O), 6.01 (3 C=O), 6.19, 6.24 ($\Delta^{1,4,6}$); found: Br, 22.13), followed by dehydrobromination with collidine.

Animal assay of V and VI showed potencies as given in the table compared to cortisone (III = 1).

	Eosinopenia[9]	Thymus Involution[9]	Liver Glycogenesis[9]
V	1	2.1	2.2
VI	1.9	2.5	4.5

The electrolyte excretion pattern using V and VI was the same as with I and II.[10] Preliminary

(1) (a) H. L. Herzog, A. Nobile, S. Tolksdorf, W. Charney, E. B. Hershberg, P. L. Perlman and M. M. Pechet, *Science*, **121**, 176 (1955); (b) H. L. Herzog, C. C. Payne, M. A. Jevnik, D. Gould, E. L. Shapiro, E. P. Oliveto and E. B. Hershberg, THIS JOURNAL, **77**, 4781 (1955).

(2) J. J. Bunim, M. M. Pechet and A. J. Bollet, *J. Am. Med. Assoc.*, **157**, 311 (1955).

(3) Meticorten, ® 1,4-pregnadiene-17α,21-diol-3,11,20-trione.

(4) Meticortelone, ® 1,4-pregnadiene-11β,17α,21-triol-3,20-dione.

(5) *Cf.* V. R. Mattox, E. L. Woroch, G. A. Fleischer and E. C. Kendall, *J. Biol. Chem.*, **197**, 261 (1952).

(6) *Cf.* W. Charney, D. Sutter, C. Federbush, M. Gilmore, H. L. Herzog, M. J. Gentles, M. E. Tully and E. B. Hershberg, to be published; T. H. Stoudt, W. J. McAleer, J. M. Chemerda, M. A. Kozlowski, R. J. Kirschmann, V. Marlatt and R. Miller, *Arch. Biochem. Biophys.*, **59**, 304 (1955).

(7) *Cf.* E. P. Oliveto, R. Rausser, L. Weber, E. Shapiro, D. Gould and E. B. Hershberg, THIS JOURNAL, **78**, 1736 (1956).

(8) C. J. W. Brooks and J. K. Norymberski, *Biochem. J.*, **55**, 371 (1953).

(9) For methods, see S. Tolksdorf, M. L. Battin, J. W. Cassidy, R. M. MacLeod, F. H. Warren and P. L. Perlman, *Proc. Soc. Exper. Biol. Med.*, **92**, 207 (1956).

(10) The biological testing was carried out by Carole Rice, Alexandra D. Stephenson, James A. Truan and Felix H. Warren. We appreciate the aid of Lawrence Finckenor, Herbert Gerber and Merl Steinberg in preparing quantities of V and VI for testing.