quickly turned red, then lightened to yellow. Removal of the phosphorus oxychloride at reduced pressure gave a glass which was treated with 5% sodium hydroxide solution to liberate the base which was then extracted with chloroform. Even under nitrogen, the chloroform solution turned bright red during the extraction; but the color could be removed by shaking with a little charcoal. After drying over sodium sulfate the solvent was removed under nitrogen.

The unsaturated base was so sensitive to oxidation that it was reduced immediately, without purification.

dl-**Alloyohimbane.** (a) **By Platinum Reduction.**—The unsaturated base from 308 mg. of *cis*-lactam was taken up in 10 cc. of absolute ethanol and hydrogenated at atmospheric pressure with 15–20 mg. of platinum oxide. Within 15 minutes 19.2 cc. of hydrogen was absorbed. The mixture was filtered, the solvent was removed and the residue was chromatographed in ether over alumina.

The reduced base appeared in the first two fractions, weighing 120 mg., and melting at 147–149° (hot-stage) after recrystallization from methanol. An analytical sample was recrystallized three more times from methanol and dried 6 hr. at 100° *in vacuo*, m.p. 143.5–144° (capillary).

Anal. Calcd. for $C_{19}H_{24}N_2$: C, 81.38; H, 8.63. Found: C, 81.44; H, 8.68.

(b) **By Reduction with Tin and Hydrochloric Acid.**—The unsaturated base from 385 mg. of the *cis*-lactam was dissolved in 15 cc. of ethanol and 10 cc. of concentrated hydrochloric acid and the mixture was refluxed gently. Over a period of 2 hr., 2.0 g. of mossy tin was added in small pieces. The solution slowly lightened from deep red to yellow. After refluxing for 2 more hr. water was added and the alcohol was removed under reduced pressure. Addition of 200 cc. of 10% sodium hydroxide solution and extraction with chloroform, followed by washing with water, drying over sodium carbonate and removal of the solvent left a residue which was chromatographed on alumina in 50/50 ether–petroleum ether. The *dl*-alloyohimbane crystallized from the second fraction, weighing 100 mg., and had m.p. 142–146°. After recrystallization from methanol it melted at 147–149° (hot-stage), alone or mixed with the base from the platinum reduction. Comparison of their infrared spectra confirmed their identity.

Comparison of both bases with racemic alloyohimbane from sempervirine was carried out, using a sample kindly furnished by Dr. Janot.[5] The reported melting point of 134° was raised after two recrystallizations from methanol to 142–146° (hot-stage). A mixture with our synthetic base, m.p. 147–149° (hot stage), had m.p. 144–148° (hot stage). The infrared spectra of the two substances were superimposable.

dl-**3-Epialloyohimbane.**—The unsaturated base from 300 mg. of the *cis*-lactam was dissolved in 250 cc. of liquid ammonia in a Dewar flask. One gram of freshly cut sodium and 3 cc. of anhydrous *t*-butyl alcohol were added in six portions over 1 hr., with a continuous stirring. After stirring for an additional hour, 0.3 g. of sodium was added and stirring was continued for another hour. Methanol was then added dropwise until the blue color disappeared and the ammonia was allowed to evaporate.

The residue was diluted with water and extracted with chloroform. After washing with water, treating with charcoal and drying over sodium carbonate, the solvent was removed, and the residue was chromatographed over 5 g. of alumina in 50/50 ether–petroleum ether. The second fraction gave 100 mg. of beautiful white needles which melted at 185–186° (hot-stage) after three recrystallizations from methanol.

Anal. Calcd. for $C_{19}H_{24}N_2$: C, 81.38; H, 8.63. Found: C, 81.66; H, 8.65.

NEW YORK 27, N. Y.

COMMUNICATIONS TO THE EDITOR

ON THE ORIGIN OF C_{28} IN ERGOSTEROL

Sir:

There is considerable evidence that the biological syntheses of ergosterol and cholesterol follow the same general plan and branch from each other only at a relatively late stage. As has been shown in a study with an acetate-less mutant of neurospora, at least 26 of the 28 carbon atoms of ergosterol stem from acetic acid.[1] The distribution of isotope in biosynthetic ergosterol is only partially known but since C_{23} and C_{25}[2,3] and C_{11} and C_{12}[4,5] are derived from acetate-carboxyl in both cholesterol and ergosterol, it seems reasonably certain that the patterns are the same in both instances. On the other hand, the origin of C_{28}, the methyl substituent in the ergosterol side-chain, has hitherto remained obscure. C_{28} does not become labeled when 1-C^{14}-acetate is the precursor of ergosterol in yeast,[3] nor is the methyl carbon of acetate utilized, at least for C_{28} of the similarly side-chain substituted eburicoic acid.[6] We now report results showing that formate is an efficient carbon source for C_{28} of ergosterol. Yeast was suspended in a medium containing non-isotopic glucose plus C^{14} formate, and the ergosterol isolated from the cells after an incubation of 24 hours. Ozonization of the sterol yielded α,β-dimethylbutyraldehyde and this was further degraded by way of methyl isopropyl ketone to afford C_{28} as iodoform.[3] The specific activities listed in the table for ergosterol, the DNP derivative of the C_6-aldehyde, the semicarbazone of methyl isopropyl ketone and for iodoform clearly show that C^{14} from formate enters only the sterol side chain and that the C^{14} content of C_{28} accounts, within the limits of error, for all the radiocarbon incorporated into ergosterol. That formate is a carbon source specifically for C_{28} is further borne out by the finding that the non-saponifiable lipids of formate-incubated cells yielded on chromatography a single radioactive peak and this coincided with the ergosterol fraction. There was no evidence for the presence of radioactive squalene, zymosterol or lanosterol, all of which are

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(5) W. G. Dauben and T. W. Hutton, THIS JOURNAL, **78**, 2647 (1956).

(6) J. H. Richards, Dissertation, University of California, 1956.

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[3] 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.

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CONVERSE MEMORIAL LABORATORY
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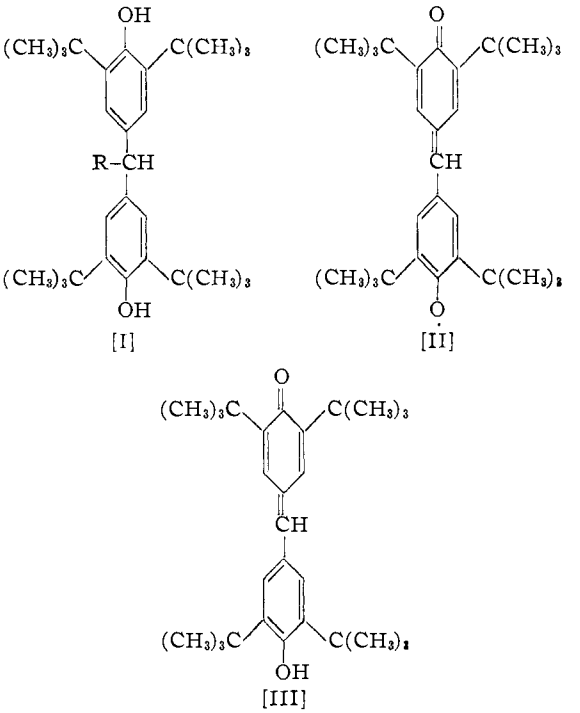
RECEIVED DECEMBER 17, 1956

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

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