the "corrected" spectra of Ia, Ib, and Ic as described in Table I. The spectral characteristics of these compounds after 1 hr. exposure to bright sunlight are also given.

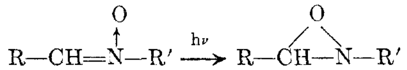
It can be seen that on irradiation the "K band" absorption has disappeared completely for all three compounds with not even an inflection to mark its former position. In addition, the following changes have taken place: In compounds Ia and Ib the "E_1 bands" have undergone slight bathochromic displacements from 232 to 235 mμ and from 237 to 240 mμ, respectively. In compound Ic the "E band" at 265 mμ persisted while that at 247 mμ was replaced by one at 237 mμ.

With the exception of a single band, Wheeler and Gore's description of the spectra is consistent with what might be expected from mixtures of the original nitrones and their irradiation products. This exception involves the peak at 280 mμ (ϵ = 7,950) for α-(*p*-anisyl)-*N*-phenylnitrone, Ib, which we were unable to observe either in the purified original nitrone or in the photochemically converted compound. The anomalous absorption may have been due to the presence as an impurity of anisaldehyde which shows λ_{max} at 276.5, ϵ_{max} = 15,500[4] and is one of the reactants used in the preparation of Ib as well as being a potential hydrolysis product.

We agree on the whole with Wheeler and Gore's interpretation of the origin of the various regions of absorption, but disagree on the assignment of various bands. The 265 mμ band of Ic, for example, is probably the displaced nitrobenzene absorption[5] and would be classified as "E_1" rather than "E_2." This assignment is strengthened by the fact that the absorption persists after irradiation when the conjugative ability of the central linkage has presumably been eliminated. The 258 mμ band for Wheeler and Gore's α-(*m*-nitrophenyl)-*N*-phenylnitrone might similarly be classified as "E_1."

The nature of the photochemical reaction which causes these changes in spectra is as yet unknown. Dimerization, polymerization, or other multimolecular reactions seem inconsistent with the rapidity with which the "K bands" disappear, the spectral change being complete in less than 1 min. at 3.00 × 10^{-5} molar concentrations. The possibility of a *cis-trans* isomerization is discounted by the fact that such a transformation would be expected to lead at most to a hypsochromic shift of 20–40 mμ rather than complete disappearance of the longer wave-length absorption.

More likely is the possibility that the reaction taking place involves conversion of the nitrone to the corresponding oxazirane, a new chemical species recently reported by Emmons.[6]



$$R{-}CH{=}\overset{\overset{O}{\uparrow}}{N}{-}R' \xrightarrow{h\nu} R{-}\overset{O}{CH}{-}N{-}R'$$

This possibility is currently being investigated and will be reported in a future publication.

EXPERIMENTAL

The nitrones were kindly furnished to us by Professor Ernest F. Pratt of the University of Maryland. They were prepared by Mr. Dawood Rejali and their syntheses will be described by Pratt and Rejali in a forthcoming publication. The compounds slowly decomposed on standing in the light and were consequently recrystallized from absolute ethanol to constant spectra and to the following melting points: Ia, 113.5–114°; Ib, 116.6–117.8°; Ic, 186–187°.

Ultraviolet spectra were measured in 1-cm. quartz cells using a Cary Model 14 spectrophotometer and checked on a Beckman DU. Solutions were 3.00 × 10^{-5} molar. In determining the "corrected" spectra all dilution operations were carried out in a darkened room using Pyrex brand low actinic volumetric glassware (Corning No. 55640).

Acknowledgments. We are grateful to Dr. E. F. Pratt for furnishing the nitrones and to Drs. Pratt, D. V. Sickman, and O. H. Johnson for helpful discussions.

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[4] A. Burawoy and J. T. Chamberlain, *J. Chem. Soc.*, 2310 (1952).

[5] Nitrobenzene shows λ_{max} at 260 mμ in ethanol. P. Fielding and R. J. W. LeFèvre, *J. Chem. Soc.*, 2812 (1950).

[6] W. D. Emmons, *J. Am. Chem. Soc.*, **78**, 6208 (1956).

Microbiological Transformation of Steroids. V. Action of Several Bacterial Species on 4-Pregnene-17α, 21-diol-3,20-dione

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In a recent patent is reported the transformation of 4-pregnene-17α,21-diol-3,20-dione 21-acetate into 1,4-pregnadiene-17α,21-diol-3,20-dione (II) and 4-pregnene-14α,17α,21-triol-3,20-dione by the action of a culture of *Mycobacterium smegmatis.*[1] *Mycobacterium lacticola* is mentioned as a member of this genus which accomplishes the same transformations.

We have observed the formation of still another product, 1,4-pregnadiene-17α,20β,21-triol-3-one (III), from 4-pregnene-17α,21-diol-3,20-dione (I) by the action of *Mycobacterium lacticola* (A.T.C.C., 9626). Isolation of III was accomplished by chromatography and subsequent formation of the 20,21-diacetate. Reduction of the 20-carbonyl in our

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[1] G. Shull and D. Kita, Belgian Patent **538,327**.

hands may possibly be accounted for by the different medium we employed for the transformation. This same effect has been noted previously by us in the action of *Cornynebacterium simplex* on I from which either II or III may be produced as the major product by appropriate choice of medium.[2]

We also observed that an unidentified species of the genus *Alcaligenes*, family Achromobacteriaceae, (Schering ON No. 1-42) converted I into III. In this instance it was possible to isolate III by chromatography without subsequent acetylation.

EXPERIMENTAL[3]

Myocobacterium lacticola. To each of ten 300-ml. Erlenmeyer flasks was added 100 ml. of a broth made from 10 g. of yeast extract (Difco), 10 g. of cerelose, 4.4 g. of potassium dihydrogen phosphate, and 8.84 g. of disodium hydrogen phosphate heptahydrate made up to 1 l. with tap water. The flasks and their contents were sterilized, inoculated with a loopful of *Mycobacterium lacticola* (A.T.C.C. 9626), and incubated, with rotary shaking at 220 cycles/min., for 48 hr. at 30°. Then, to each flask was added 0.025 g. of I in 0.5 ml. of 80% aqueous ethanol. Shaking was continued for 48 hr. at which time extraction of an aliquot of the reaction mixture with chloroform followed by chromatography on Whatman No. 4 paper by the method of Shull[4] indicated complete transformation of I. A single, more polar, spot absorbing in the ultraviolet, was observed, which did not stain with red tetrazolium. The total reaction mixture was extracted with chloroform and the extracts were concentrated to a small volume which was chromatographed over 20 g. of Florisil prepared with hexane. Elution with methylene chloride containing 0.5%, 1.0%, and 2.0% of methanol afforded a series of glassy residues which appeared to be paper chromatographically homogeneous. They were pooled and acetylated with 2 ml. of acetic anhydride in 2 ml. of pyridine. After 16 hr. at room temperature, 50 ml. of water was added and the oily precipitate was allowed to stand in contact with the solvent until crystallization ensued (*ca.* 1 month). The resulting prisms were removed by hand and washed with cold ether. A total of 0.040 g. of solid, m.p. 175-177°, was collected. Bands were observed in the infrared spectrum of this compound at 2.87μ (OH), 5.75μ (acetate carbonyl), 6.01, 6.14 and 6.22μ (1,4-diene-3-one) and 8.16μ (C—O—C of acetate). The spectrum was identical with that from the 20,21-diacetate of III.[2] Admixture with an authentic sample did not depress the melting point.

Alcaligenes sp. (ON No. 1-42). To each of ten 300-ml. Erlenmeyer flasks was added a broth made from 3 g. of yeast extract (Difco), 10 g. of cerelose, and 1 g. of corn steep liquor per liter of tap water. The pH was adjusted to 7.0 and the sterile medium was inoculated with a 5% charge of *Alcaligenes sp.* (ON No. 1-42) (20-hr. growth culture). After 24 hr. of incubation, under the conditions described previously, 0.025 g. of I in 2 ml. of 95% ethanol was added to each flask and incubation with shaking was continued for 72 hr. The reaction mixture was extracted with chloroform, washed with water, dried, and concentrated, and the residues were chromatographed on 15 g. of Florisil in the usual way. Crystalline III was obtained from the 1% methanol in methylene chloride eluates. Recrystallization from acetone-hexane afforded 65 mg. of III, m.p. 190-193°, $\lambda_{\max}^{\text{Nujol}}$ 3.02μ (OH), 6.02, 6.19, and 6.24μ ($\Delta^{1,4}$-diene-3-one). The infrared spectrum was identical with that from an authentic sample.[2]

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[2] H. L. Herzog, C. C. Payne, M. E. Tully, M. A. Jevnik, E. B. Hershberg, A. Nobile, W. Charney, C. Federbush, D. Sutter, and P. L. Perlman, unpublished results; also H. L. Herzog, Gordon Conference on Steroids and Natural Products, August 1955.

[3] Infrared measurements were made and interpreted by the Physical Chemistry Laboratory of the Schering Corp.

[4] G. M. Shull, Abstracts of the 126th Meeting of ACS, New York, 1954, p. 9A.

2-[(2-*N*-methyl-*N*-methoxyaminoethyl)-(*p*-methoxybenzyl)Amino]Pyridine. An Hydroxylamine Analog of Pyrilamine

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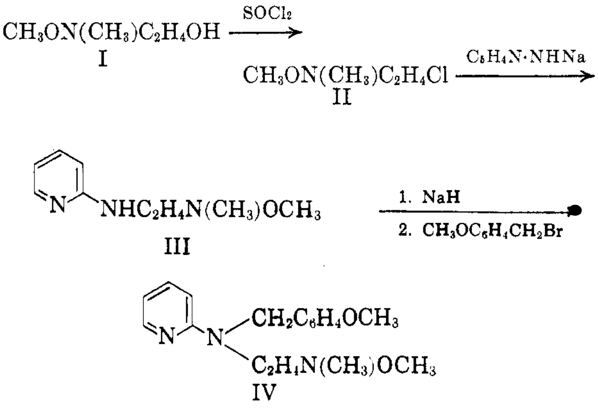
Some *O,N*-substituted hydroxylamines which are chemically related to pharmacologically active amines have pharmacological action similar to the corresponding amines.[1-3] It became of interest to determine whether the hydroxylamine analog of pyrilamine would have pharmacological properties similar to the well known antihistamine. Therefore, a sample of 2-[(2-*N*-methyl-*N*-methoxyaminoethyl)(*p*-methoxybenzyl)amino]pyridine was prepared, according to the following series of reactions:



N-2-Hydroxyethyl-*O,N*-dimethylhydroxylamine (I) was converted into *N*-2-chloroethyl-*O,N*-dimethylhydroxylamine (II) with thionyl chloride through the hydrochloride. *N*-2-Choloroethyl-*O,N*-dimethylhydroxylamine was then interacted with the reaction product of 2-aminopyridine and sodium hydride in 1,4-dioxane. The resulting product, 2-[(2-*N*-methyl-*N*-methoxyaminoethyl)amino]pyridine (III) was then treated with a suspension of sodium hydride in 1,4-dioxane. The solution, which resulted from this reaction, was treated with *p*-

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[1] L. W. Jones and R. T. Major, *J. Am. Chem. Soc.*, 49, 1527 (1927).

[2] E. F. Rogers, G. Bovet, V. G. Longo, and G. B. Marini-Bettolo, *Experientia*, 9, 260 (1953).

[3] G. Palazzo, E. F. Rogers, and G. B. Marini-Bettolo, *Gazz. Chim. Ital.*, **84**, 915 (1954).