TABLE II

	Average R_f in			UV Light			Color Reactions		
Alkaloids	C_6H_6	$CHCl_3$	BuOH AcOH	Fluorescence	Absorption	λ_{max}, mμ	H_2SO_4	Mandelin	$SbCl_5$-H_3PO_4 → H_2SO_4
Lycorine	Line	Line	0.59	Blue	–	234–238, 292	Wk. yellow	–	Yellow → Orange
A	0.36	0.71	0.64	Yellow	–	236, 286, 305	Wk. red	–	Yellow → Yellow
B	0.47	0.78	0.82	Pink	–	238, 289	Wk. yellow	–	– → Red
C	0.90	0.87	0.94	Blue	–	292	Red	–	Yellow → Orange

The extraction of fresh bulbs of *Crinum latifolium* L. with ethanol, followed by conventional separation of the total basic material gave a crude alkaloidal fraction, 0.2% by weight[7] which yielded on repeated crystallization from methanol *ca.* 0.05% of lycorine, m.p. 265–270°, found to be identical with an authentic specimen. The noncrystalline mother liquors first chromatographed in benzene showed the presence of three clearly separated compounds; the fourth compound remained on the line of application. The compounds were eluted with ethanol from the paper and again chromatographed in the butanol–acetic acid system. The least polar compound was shown to be lycorine whereas the three other substances could not be correlated with any of the samples available to us. The observed ultraviolet absorption maxima of compound A seem to indicate that this alkaloid may contain a lactone grouping[8] but naturally no definite conclusion can be drawn from the available data.

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Melting points. The melting points were determined with the Fisher-Jones melting point apparatus and are uncorrected.

Absorption spectra. Ultraviolet absorption spectra were determined in 95% ethanol with a Beckman Model DU spectrophotometer; the fluorescence in ultraviolet light was determined with a portable lamp, long wave ultraviolet Model SL 3660. All the ultraviolet light absorptions were determined on samples eluted from an untreated paper.

Chromatography. The paper chromatograms were run on a formamide treated Whatmann No. 1 paper in all the solvents except the butanol–acetic acid system which was run on untreated paper; all the solvents were purified prior to use. The butanol–acetic acid system was prepared as follows: 40 ml. 1-butanol and 10 ml. acetic acid were saturated with 50 ml. water and 5 ml. of propylene glycol was added to the organic phase. The time in which the chromatograms were run varied between 3 and 8 hours depending on the mobile phase used.

Color reactions. Reagents for the color reactions were prepared in the usual manner. The antimonium pentachloride–phosphoric acid reagent was made by dissolving 5.0 g. antimony pentachloride in 20 ml. of syrupy phosphoric acid; this solution was applied to the paper and the developed color was observed after 5 min.; the paper was then treated with concentrated sulfuric acid and the change in color was noted.

Isolation procedure. Fresh bulbs (600 g.) of *Crinum latifolium* L. collected near Lonavla, Bombay State, in May 1952 were extracted exhaustively with ethanol *in situ;* the solvent was removed by distillation under reduced pressure and the dark gummy material was dissolved in a chloroform-ethanol (3:1) mixture. The basic material was then extracted several times with 3% aqueous sulfuric acid; the combined extracts were made alkaline with dilute ammonia and the organic material was extracted with chloroform. Evaporation of the solvent gave 1.3 g. of noncrystalline material which yielded, after several crystallizations from methanol, 270 mg. of crystalline material, showing a m.p. 265–270°, no depression on admixture with an authentic specimen of lycorine.

Anal. Calcd. for $C_{16}H_{17}NO_4$: C, 66.88; H, 5.96. Found: C, 66.71; H, 6.12.

The resulting oily mother liquors were chromatographed by the descending method on a strip of paper and revealed the presence of four compounds; the strips were cut, the material was eluted with methanol and rechromatographed in the solvents as indicated in Table II.

The least polar compound was shown to be lycorine by comparing the R_f values in various solvents, general color reactions and the ultraviolet absorption, found to be 292 mμ, shoulder at 234–238 mμ; a sample of authentic lycorine showed an ultraviolet absorption maximum at 292 mμ (log ε 3.66) and a shoulder at 232–236 mμ (log ε 3.55).

Acknowledgment. The authors wish to thank Mr. E. Avila for the determination of the ultraviolet absorption spectra, Prof. H. G. Boit for samples of crinine, crinidine, lycorine, narcissidine and tazettine, and Dr. W. C. Wildman for the very generous supply of the remaining alkaloids and to H. Santapau, S.J. for the identification of the plant material.

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(7) Extraction of bulbs of *Crinum asiaticum* L. gave almost identical results.

(8) C. K. Briggs, P. F. Highet, R. J. Highet, and W. C. Wildman, *J. Am. Chem. Soc.*, **78**, 2899 (1956).

Absorption Spectra of the Phenylnitrones

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Received November 2, 1956

Wheeler and Gore[1] have recently recorded the ultraviolet absorption spectra in absolute ethanol of a number of phenylnitrones, I. Three distinct regions of intense absorption, labeled the E_1, E_2, and K bands, respectively, were observed at *ca.* 230, 280, and 320 mμ. The "E_1 bands" were at-

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(1) O. H. Wheeler and P. H. Gore, *J. Am. Chem. Soc.*, **78**, 3363 (1956).

TABLE I

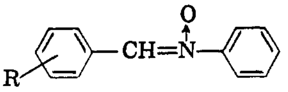
ABSORPTION SPECTRA OF PHENYLNITRONES[a]

Nitrone	Band	Wheeler and Gore[b]	This Investigation[c] "Corrected Spectra"[d]	This Investigation[c] After Irradiation[e]
Ia	E_1	227 (9,850) 236 (9,060)	232 (10,800)	235 (10,100)[f]
	K	315 (14,000)	314 (20,300)	Disappears
Ib	E_1	227 (9,800)	237 (11,800)	240 (16,700)
	E_2	280 (7,950)	Absent	Absent
	K	330 (11,800)	329 (26,900)	Disappears
Ic	E_1	228–36 (8,500)	Absent	237 (11,900)
	E_2	251 (8,470)	247 (8,600)[f]	Disappears
		266–78 (10,400)	265 (9,500)	265 (11,800)
	K	350 (10,400)	352 (20,000)	Disappears

[a] Absorption maxima (λ) in mμ; molar absorptivities (ε) in parentheses. [b] Absolute ethanol solvent. [c] Methanol solvent. [d] Dilutions carried out in dark and in protective, "low actinic" glassware. [e] Solutions 3.00 × $10^{-5}M$, exposed to bright sunlight for 1 hr. [f] Shoulder.

tributed to electronic transitions in the individual benzene nuclei, the "E_2 bands" to transitions involving the central linkage and one aromatic ring, and the "K bands" to electronic transitions through the entire molecule.

In a study of the spectral characteristics of the azomethine linkage we had previously had occasion to measure the spectra in methanol of several of these compounds, namely α,*N*-diphenylnitrone, Ia, α-(*p*-anisyl)-*N*-phenylnitrone, Ib, and α-(*p*-nitrophenyl)-*N*-phenylnitrone, Ic.



I, (a) R = H, (b) R = *p*-OCH_3, (c) R = *p*-NO_2

Comparison of our results with those of Wheeler and Gore showed the following discrepancies which seemed greater than would be anticipated only on the basis of the solvent difference: (a) While λ_{max} for the "K bands" were about the same, our values of ϵ_{max} were 40 to 80% higher; (b) Both λ_{max} and ϵ_{max} for the shorter wave-length bands differed by quantities which seemed greater than experimental error; (c) Wheeler and Gore reported a high intensity "E_2 band" at 280 mμ for Ib and an "E_1 band" at 228–236 mμ for Ic, neither of which we were able to observe, even as inflections.

In an attempt to resolve these differences, Ia, Ib, and Ic were carefully purified and their spectra redetermined in absolute ethanol. As before, values of ϵ_{max} for the longer wave-length bands differed substantially from those reported by Wheeler and Gore but, in addition, differences of as much as 10% were observed between our own ostensibly replicate determinations. Such variations were, of course, greater than normally considered acceptable for precise spectral work.

Earlier experiences of a similar nature with the β-nitrostyrenes[2] led us to suspect that alteration of the spectra was being caused by some sort of photochemical reaction. This was confirmed when a 3.00 × 10^{-5} molar solution of Ia in methanol, which initially had an absorbance of 0.609 at 314 mμ, was exposed to bright sunlight for several seconds. The absorbance dropped to 0.450 and after an additional several seconds to less than 0.300. One minute in bright sunlight eliminated this "K band" absorption completely. By comparison, the spectrum of a similar solution which had been allowed to stand in the dark for several days was identical with that originally obtained. Compounds Ib and Ic acted similarly. It appears, therefore, that the lowered values of ϵ_{max} for the "K bands" and the displacement of the "E_1 and E_2 bands" in the spectra described by Wheeler and Gore for Ia, Ib, and Ic resulted as a consequence of this photochemical transformation which, in very dilute solutions, takes place quite rapidly in a normally lighted room.[3]

These findings emphasize the care that must be taken to guard against such photochemical effects in ultraviolet spectrophotometric work. For this reason we have in these laboratories adopted the routine procedure of remeasuring the spectra of all new compounds after first exposing the diluted solutions to sunlight for several minutes. Where compounds are found to be photosensitive, manipulations are carried out in a darkened room and protective "low actinic" glassware is used for all dilutions.

These precautions, which generally give accurate reproducible spectra, were observed in determining

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(2) M. J. Kamlet and D. J. Glover, *J. Am. Chem. Soc.*, **77**, 5696 (1955).

(3) It should be noted that similar inaccuracies in the spectra reported by Wheeler and Gore for their other ten nitrones would explain the seeming anomalies in the angles of deformation from planarity which they calculated from the equations $\cos \theta_1 = \epsilon/\epsilon_0$ [E. A. Braude, W. F. Forbes, and F. Sondheimer, *Nature*, **173**, 117 (1954).] and $\cos^2 \theta_2 = \epsilon/\epsilon_0$ [H. B. Klevens and J. R. Platt, *J. Am. Chem. Soc.*, **66**, 1714 (1944)] where ε is the molar absorptivity of the *ortho*-substituted nitrone and ϵ_0 that of the corresponding *para*-derivative.

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.

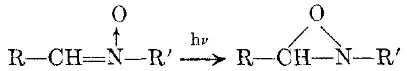
It can be seen that on irradiation the "K band" absorption has disappeared completely for all three compounds with not even an inflection to mark its former position. In addition, the following changes have taken place: In compounds Ia and Ib the "E_1 bands" have undergone slight bathochromic displacements 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]



$$R\text{—}CH{=}\overset{\overset{O}{\uparrow}}{N}\text{—}R' \xrightarrow{h\nu} R\text{—}\overset{O}{\overbrace{CH\text{—}N}}\text{—}R'$$

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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Received November 5, 1956

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**.