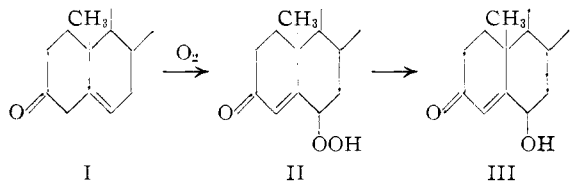
oxidation of cholesterol. None of the products administered as aqueous colloids have had a carcinogenic effect, although a number of them administered in sesame oil have produced positive results, indicating that the presence of sesame oil is an essential link in the carcinogenic chain.[6] As recently reported,[7] cholesterol α-oxide, Δ^4-cholestene-3,6-dione, and the 1:1 complex[5] of 6β-hydroxy-Δ^4-cholestene-3-one and epicholesterol when injected into mice in sesame oil produced fibrosarcomas in 19–43% of the mice at the average age of 13–14 months. These compounds produced negative results when administered as aqueous colloids.

We wish now to report discovery of a still more potently carcinogenic oxidation product, Δ^5-Cholestene-3-one, an easily isomerized and highly reactive substance that is oxidized by lead tetraacetate to the 4α-acetoxy derivative,[8] combines with molecular oxygen in hexane solution at 25° to give a compound that crystallizes from methanol in needles, m.p. 180°, λ^{EtOH} 236 mμ (E = 16,850).

Anal. Calcd. for $C_{27}H_{44}O_3$ (416.62): C, 77.83: H, 10.65. Found: C, 77.82: H. 10.72. The substance gives an immediate color with sodium iodide–acetic acid (thiosulfate titer: mol. wt. 425) and is reduced to 6β-hydroxy-Δ^4-cholestene-3-one (III),



I II III

m.p. 193°, a_D + 27.2° Chf, mixed m.p. 193–194°. Since the ultraviolet absorption corresponds to that of III, and since reduction of hydroperoxides is known to cleave the oxygen–oxygen bond, the substance is identified as 6β-hydroperoxy-Δ^4-cholestene-3-one (II).

The hydroperoxide II was given in three spaced subcutaneous injections of 5 mg. each in sesame oil to each of 32 Marsh-Buffalo mice.[9] At the age of 12 months, fibrosarcomas have appeared at the site of injection in 13 of the mice treated (average tumor age 9.6 mo.), and 17 of the remaining mice were still alive. No fibrosarcomas were observed in litter-mates given the same amount of II in aqueous colloidal solution, nor in a series of controls (up to 18 months of age) which received only sesame oil.

Indirect evidence that the precursor I can be formed from cholesterol in the body is afforded by isolation of probable transformation products: Δ^4-cholestene-3-one,[10] $\Delta^{4,6}$-cholestadiene-3-one[11] (swine spleen, arteriosclerotic aorta), and coprostanone[12] (ambergris). The possibility that antioxidants may increase resistance to spontaneous carcinogenesis is under investigation.

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RECEIVED JUNE 24, 1955

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POLARIZATION OF THE 1850 Å. BAND OF AMIDES[1]

Sir:

We have recently carried out a measurement of the direction of the transition moment vector for the first N,V transition in amides.[2] Observations were made on sublimation flakes of myristamide[3] ($C_{13}H_{27}CONH_2$) with light polarized along the a and b crystallographic axes, which are also the principal directions, in the region between 2300 and 1600 Å. The orientation of samples in these experiments was obtained crystallographically with the aid of X-ray precession photographs. The ratio of the optical densities observed along the principal directions over the long-wave-length one-third of the main band was found to be constant at D_a/D_b = 14.62. This gives for the orientation of the electric moment $\theta = \pm$ arctan 0.262 where θ is measured from the a axis. The two orientations referred to the amide group itself were computed from the known crystal structure of myristamide[3] and are 9.1° and 26.7° from the line joining the nitrogen and oxygen (see Fig. 1).

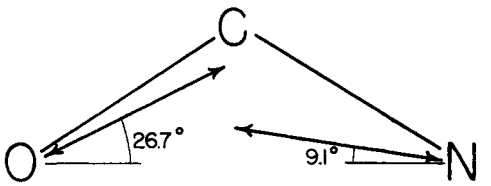

Fig. 1.—Possible orientations of the electric vector for the 1850 Å. electronic absorption band in amides.

Neither of these values agrees with conclusions from a recent experiment[4] in which absorption at 2100 Å. was found to occur essentially along one of the principal directions (also here the c crystallographic axis) of crystalline N-acetylglycine. In that work it was inferred that absorption must be nearly along the carbon–nitrogen line of the amide group, a line which is only four degrees from one of the principal directions. Possibly what was actually measured was mainly a single allowed crystal transition occupying a small region at the extreme long-wave-length edge of the main absorption. Such absorption would have to be along one or the other principal direction. Indeed we have found absorption in the region from 2120 to 2250 Å. at the long-wave-length edge of the band for myristamide which is, within experimental error, exactly along the a axis, a principal direction. In addition, we

(1) Supported in part by the Air Research and Development Command, Contract No. AF 18(600)-375.

(2) J. S. Ham and J. R. Platt, *J. Chem. Phys.*, **20**, 335 (1952); and H. D. Hunt and W. T. Simpson, THIS JOURNAL, **75**, 4540 (1953).

(3) The crystal structure of myristamide was determined by J. D. Turner, Ph.D. Thesis, University of Washington, 1953, and refined by R. F. Adamsky, private communication.

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have made theoretical calculations of the $1/R^3$ force modifications of the spectrum to be expected for myristamide (using both of the angles given in Fig. 1). These calculations support the interpretation of the 2120–2250 Å. absorption as a crystal transition, in that the long-wave-length allowed component is predicted to occur in the *a* axis direction, split off by *ca.* 100 to 150 Å., just as observed (and regardless of which angle is chosen).

We have also made quantum-theoretical calculations of the direction of the transition moment for an amide model. In the model an amide is regarded as a perturbed allyl anion[5] with the nitrogen represented by a deepening of the coulomb potential on one of the end carbon atoms and the oxygen considered as being the same as carbon. The result is that the direction should be close to the nitrogen–oxygen line, but inclined toward the carbon-nitrogen line. Of the two values found in our experiments the one with an angle of 9.1° from the nitrogen–oxygen line is thus favored. Both the experimental and theoretical aspects of this investigation will be reported in detail in a forthcoming paper.

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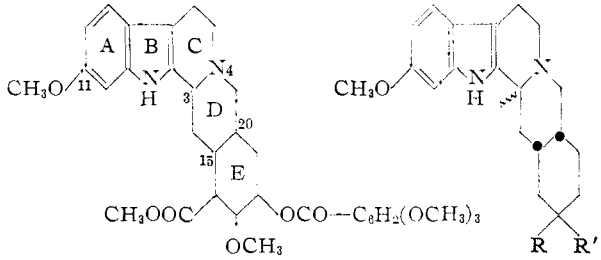
(5) Calculation was facilitated by use of full configuration interaction A.S.M.O. calculations on allyl anion (H. D. Hunt, D. L. Peterson and W. T. Simpson, to be published).

(6) National Science Foundation Predoctoral Fellow, 1954–1955.

THE D/E *CIS* RING JUNCTURE OF RESERPINE

Sir:

Reserpine, the potent hypotensive agent obtained from various species of Rauwolfia, has been assigned the structure I.[1,2] Because of its proxi-



I IIa, b

mate relation to deserpidine (formula I with the 11-methoxyl replaced by —H), Schlittler, *et al.*, surmised that reserpine too possesses a D/E *cis* (allo) ring juncture[3]; and Wintersteiner, *et al.*,[4] on the basis of the intramolecular N-4 quaternization of methyl reserpate tosylate, inferred the same relationship. We wish to report for this view conclusive confirmation obtained through an 11-methoxy-alloyohimbane (IIa, R = R′ = H) (reserpane) synthesis which is stereochemically unambiguous insofar as the C_{15}-C_{20} asymmetric centers are concerned.[5]

6-Methoxytryptamine[6] (obtained through the route: 6-methoxyindole → 6-methoxygramine methosulfate → 6-methoxyindoleacetonitrile → 6-methoxytryptamine) was alkylated in boiling dimethylformamide by ethyl *dl-cis*-2-bromomethylcyclohexaneacetate,[7,8] affording the lactam of *dl-cis*-N-(β-3′-indolylethyl)-2-aminomethylcyclohexaneacetic acid (III) (benzene solvate), m.p. 72.5–74.0° (Calcd. for $C_{20}H_{26}N_2O_2 \cdot C_6H_6$: C, 77.19; H, 7.97. Found: C, 77.02; H, 7.95). Heating III with phosphorus oxychloride in benzene, followed by platinum-catalyzed reduction of the unisolated Δ^3-ring-closed product, yielded the desired *dl*-allo base IIa, which melted at 209–210° after crystallization from methanol. Infrared spectral comparison of chloroform solutions of IIa and a reserpane derived by reduction from reserpone (IIb, R, R′ = O)[9] showed the two substances to be, apart from the racemic nature of the former, identical.[10]

Publication of our views on the nature of the remaining asymmetric centers in reserpine and deserpidine, including evidence relating thereto, is anticipated.

Acknowledgment.—The authors wish to express their gratitude to the Department of Health, Welfare and Education for financial support (Grant No. G-3892) and to S. B. Penick and Co. for a gift of reserpine.

(5) The matter of the stereochemistry at C_3 in our synthetic product is deferred for the present.

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(10) We should like to thank Dr. Schlittler and Dr. St. André for their assistance in establishing the identity of the two specimens. In addition, our base IIa was shown to be identical with material obtained by them *via* a different synthetic route.

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POLY-GLUTAMYL PTERIDINE COENZYMES

Sir:

It was recently demonstrated that the conversion of serine to glycine by a bacterial extract is dependent upon DPN, Mn^{++}, pyridoxal phosphate, orthophosphate and catalytic levels of a new coenzyme, Co C.[1,2] Co C is isolated from *Clostridium cylindrosporum*, and substitutes for but is not identical with known folic acid derivatives. By means of fractional acetone precipitation, chromatography on cellulose columns, and repeated paper chromatography in various solvent systems,[3] five groups of pteridine derivatives with Co C activity have been separated in relatively pure form from extracts of

(1) L. Dorfman, A. Furlenmeier, C. F. Huebner, R. Lucas, H. B. MacPhillamy, J. M. Müller, E. Schlittler, R. Schwyzer and A. F. St. André, *Helv. Chim. Acta*, **37**, 59 (1954).

(2) N. Neuss, H. E. Boaz and J. W. Forbes, THIS JOURNAL, **76**, 2463 (1954).

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