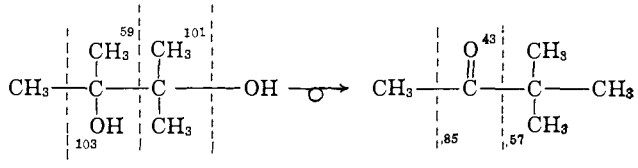
In the mass spectra of N,N-diphenylphenylacetamide and N,N-ditolylphenylacetamide, peaks corresponding to $C_6H_5NH_2$, m/e = 93, and $CH_3C_6H_4NH_2$, m/e = 107, were observed.

A comparison of the relative intensities of the peak at m/e = 208 in the spectra of N,N-ditolylphenylacetamide and IV, respectively, showed that as expected from chemical analogy[8] *p*-tolyl (~1.0% Σ_{50}) is a better migrating group than phenyl (~0.2% Σ_{50}). Other examples of migration of aryl and alkyl groups taking place under electron impact have also been observed. We suggest that these rearrangements parallel the Beckmann and pinacol–pinacolone rearrangements known to take place in solution. In the mass spectrometer, however, no solvent is present nor is there a possibility for anything except intramolecular rearrangements.[9]

Thus, the mass spectrum of benzophenone oxime shows a peak corresponding to m/e = 105 (~4.0% Σ_{40}) which can best be explained as the fragment $C_6H_5C{=}O$ to be expected from the Beckmann rearrangement product phenylacetanilide. The metastable peak at m/e = 56.5 supports this rearrangement (calcd. 56.0 for m/e = 197 → 105). As a matter of fact, a direct comparison has shown that the spectrum of benzophenone oxime contains all the peaks found for phenylacetanilide. Analogous results have been obtained with the oximes of acetophenone and *p*-tolyl methyl ketone.

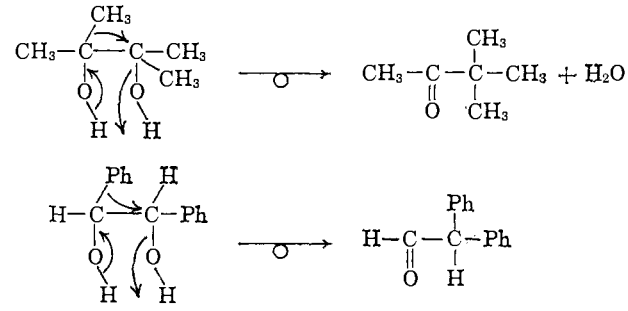
The mass spectrum of pinacol (VI) shows peaks corresponding to M − 18, 43 (~0.8% Σ_{40}), 57 (~2.5% Σ_{40}), and 85 (~1.6% Σ_{40}). These observations can be satisfactorily accounted for on the basis of the pinacolone rearrangement under electron impact.[10] Deuteration studies support this.



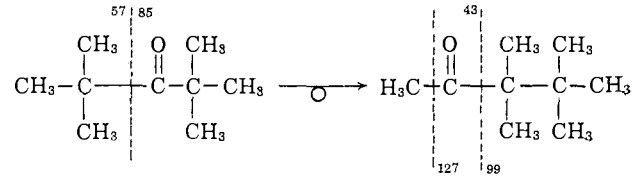
VI + H_2O

Rearrangement of hydrobenzoin (VII) to diphenylacetaldehyde (VIII) through a phenyl migration has to be assumed to account satisfactorily for the mass spectrum of hydrobenzoin.

A six-centered mechanism without invoking a carbonium ion can be written for the pinacol–pinacolone and similar rearrangements.



It has been reported[11] that di-*t*-butyl ketone (IX) rearranges in the presence of sulfuric acid to methyl heptyl ketone (X). The mass spectrum of IX does show peaks at m/e = 99 (~0.2% Σ_{40}) and 43 (~1.2% Σ_{40}) indicating alkyl migration under electron impact.[12]



IX X

Studies on isotope-labeled compounds and work on other types of rearrangement is in progress.

Acknowledgment.—This work was supported in part by a grant (MH-03930) from the U. S. Public Health Service. We are grateful to Dr. J. H. Davis and Dr. L. Z. Pollara for their active interest.

(11) J. Hine, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p. 333.

(12) At this stage it is not possible to exclude other mechanisms to account for some of the peaks in the mass spectra of VI and IX.

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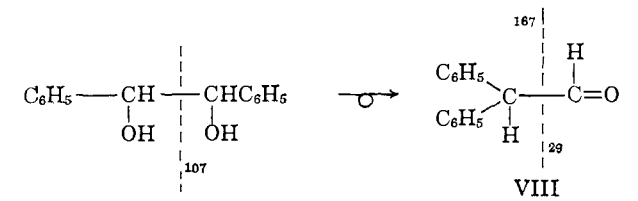


VII VIII

(8) H. Gilman, "Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1938, p. 275; see also G. W. Wheland, "Advanced Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1960, p. 593.

(9) All the spectra were recorded on a type 21-103C mass spectrometer operating at 70 e.v. using an all-glass inlet system heated to temperatures well below the melting point of samples to minimize thermal breakdown. The compounds used did not contain any of the suggested rearrangement products as contaminants.

(10) The possibility of thermal rearrangement can be discounted because the pinacol–pinacolone rearrangement takes place thermally only at high temperatures in the presence of alumina; see G. W. Wheland, "Advanced Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1960, p. 537.

Concerning the Nonphotochemical Biosynthesis of Vitamin D_3 in Fish[1]

Sir:

The pioneering work of Bills[2] on cod and catfish showed that neither irradiation nor diet allowed an adequate explanation for the origin of vitamin D in the fish studied, and measurements by Hulburt[3a] and by Atkins and Poole[3b] have indicated that sea water

(1) Supported in part by grants from the American Cancer Society (No. P-292) and the National Institutes of Health (No. CRTY-5001). W. R. N. wishes to acknowledge a stimulating discussion on the origin of the vitamins D which he had in 1958 with Dr. Konrad Bloch. This discussion formed part of the impetus to undertake this investigation. We are also most grateful to Mr. Charles Wheeler of the U. S. Fish and Wildlife service and Dr. John S. Rankin of the University of Connecticut for supplying live Atlantic bass.

(2) C. E. Bills, *J. Biol. Chem.*, **72**, 751 (1927).

(3) (a) E. O. Hulburt, *J. Opt. Soc. Am.*, **17**, 15 (1928); (b) W. R. G. Atkins and H. H. Poole, *Trans. Roy. Soc.* (London), **B222**, 129 (1933).

absorbs all but traces of irradiation near 300 mμ in the first few meters.[4] Although a number of parameters in these experiments were left undefined, they strongly suggest, as Bills[2,5] pointed out, that fish have the ability to produce vitamin D by a nonphotochemical process. We should like to present further evidence in support of this idea.

4-C^{14}-7-Dehydrocholesterol[6] (2.2×10^7 counts/min.; 8.6×10^6 c.p.m./mg.) was incubated in the dark with 20 ml. of a homogenate[7] of the livers of 2-year-old Atlantic striped bass. To the homogenate was then added 2.0 mg. of carrier vitamin D_3, and the mixture was saponified in darkness under nitrogen. The hexane-soluble material was chromatographed on silicic acid, and vitamin D_3 was apparent by ultraviolet assay in a separate band eluted just prior to cholesterol. The fractions containing the vitamin were radioactive (3.0×10^4 c.p.m.), and the peak of radioactivity coincided with the peak of λ_{max} 264 mμ. Most of the material (2.7×10^4 c.p.m.) from the combined fractions together with 1.6 mg. of additional carrier was submitted to g.l.c. at 230° on nitrile silicone gum (XE-60) deposited on silanized diatomaceous earth. As observed by Ziffer, *et al.*,[8] the mass determination showed conversion *in situ* to pyro- and isopyrocholecalciferol. The effluent was collected in fractions, and radioactivity was found with peaks coinciding with the two transformation products (2.9×10^3 c.p.m. and 2.5×10^3 c.p.m., respectively). In addition, 7.0×10^3 c.p.m. was removed in the solvent region, 980 c.p.m. between the two transformation products, and 2.7×10^3 c.p.m. in a "tail," and 1×10^4 c.p.m. was not recovered at all. The material corresponding to the two products was esterified with 3,5-dinitrobenzoyl chloride and cocrystallized to constant specific activity with authentic samples of the two dinitrobenzoates prepared by a modification of the procedure of Windaus, *et al.*,[9,10] and 76% and 52%[11] of the label was retained, respectively, in the two cases. The ratio of radioactivities in the two products (calculated from the final specific activities and the amount of carriers used) was the same (pyro/isopyro = 1.8) within reasonable experimental error as that obtained in the masses (1.8) in the radioactive case and in the masses (1.9) in a standard, nonlabeled case.

(4) Deeper penetration has been claimed by N. G. Jerlov, *Nature*, **166**, 111 (1950), but see ref. 5a, p. 171.

(5) For a review of this problem, see (a) C. E. Bills, "The Vitamins," Vol. II, W. H. Sebrell, Jr., and R. S. Harris, Ed., Academic Press, Inc., New York, N. Y., 1954, pp. 132–209; (b) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Company, New York, N. Y., 1959, pp. 90–168.

(6) Prepared according to a modification of the procedure of B. D. Kulkarni, G. Blondin, and W. R. Nes, *Steroids*, **1**, 21 (1963). Assay by the dibromide technique indicated 2.0% cholesterol as a contaminant.

(7) The homogenate was prepared in phosphate buffer, pH 7.4, and contained nicotinamide, EDTA, GSH, and ATP. It was not fractionated by centrifugation.

(8) H. Ziffer, W. J. A. VandenHeuvel, E. O. A. Haahti, and E. C. Horning, *J. Am. Chem. Soc.*, **82**, 6411 (1960).

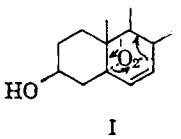
(9) A. Windaus, M. Deppe, and W. Wunderlich, *Ann.*, **533**, 118 (1938).

(10) A. Windaus, M. Deppe, and C. Rosen-Runge, *ibid.*, **537**, 1 (1939).

(11) The low recovery (52%) of radioactivity in the case of isopyrocholecalciferol may be due to the presence of labeled cholesterol which has the same g.l.c. retention time within 5%. Due to the relatively large amount of vitamin D during g.l.c., cholesterol would not be detectable in the mass. Cholesterol was in the original substrate and was also formed from it during incubation, and some of the leading edge of the cholesterol band in the silicic acid column may have been present in the vitamin band. We were able to show, however, that, as the 3,5-dinitrobenzoates, cholesterol and isopyrocholecalciferol do not cocrystallize.

(12) W. R. Nes, G. Blondin, J. L. Scott, J. K. Hummer, and B. D. Kulkarni, *Federation Proc.*, **22**, Part I, 593 (1963).

We have previously reported[12] that 4-C^{14}-7-dehydrocholesterol leads in similar dark incubations to a metabolite which upon esterification with 3,5-dinitrobenzoyl chloride gives a radioactive substance which cocrystallizes with vitamin D_3 3,5-dinitrobenzoate. In neither the latter nor the presently reported experiments did control incubations (lacking tissue or with boiled tissue) lead to labeled material which cocrystallized with the dinitrobenzoate of vitamin D_3. Consequently, on the *assumption* that a contaminant would not have remained throughout all the procedures now used, we believe it probable that 7-dehydrocholesterol was converted nonphotochemically to vitamin D_3. If this is so, a reasonable intermediate on chemical grounds could be the 5,8-peroxide of 7-dehydrochlolesterol. Such peroxides of $\Delta^{5,7}$-steroids have already been isolated from mammalian liver[13] as well as from a mold[14] grown in darkness, and more recently we have found[15] that 7-dehydrocholesterol leads to its 5,8-peroxide in fish liver homogenates incubated in the dark. At least two mechanisms are available for the peroxide to proceed to the intermediate previtamin D described earlier by Velluz and his associates.[16] A direct route indicated by I consists of a recycling of the electrons



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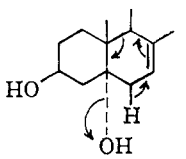

II

with elimination of oxygen. This route, however, obviously does not allow for an input of energy in going from 7-dehydrocholesterol to the secosteroid. This would be feasible by reduction of the peroxide to the Δ^7-3β,5α-diol, which has been found[17] to arise nonbiologically by hydride attack, followed by elimination of the tertiary hydroxyl group as indicated by II. The latter route would involve not only the electron transport system in the reduction, but also could reasonably involve the conversion of ATP to ADP and phosphate ion during the last step in the same way that Bloch and his associates[18] have found for mevalonic acid, which undergoes elimination of the tertiary hydroxyl group with cleavage of the α,β-bond.

(13) D. Dvornik, M. Kraml, J. Dubuc, M. Givner, and R. Gaudry, *J. Am. Chem. Soc.*, **85**, 3309 (1963).

(14) P. Wieland and V. Prelog, *Helv. Chim. Acta*, **30**, 1028 (1947).

(15) B. D. Kulkarni, G. A. Blondin, and W. R. Nes, unpublished observations.

(16) L. Velluz, G. Amiard, and A. Petit, *Bull. soc. chim. France*, **16**, 501 (1949); L. Velluz and G. Amiard, *ibid.*, **22**, 205 (1955); L. Velluz, G. Amiard, and B. Goffinet, *ibid.*, **22**, 1341 (1955). See also A. L. Koevoet, A. Verloop, and E. Havinga, *Rec. trav. chim.*, **74**, 788 (1955).

(17) G. F. Laws, *J. Chem. Soc.*, 4185 (1953).

(18) M. Lindberg, C. Yuan, A. deWaard, and K. Bloch, *Biochemistry*, **1**, 182 (1962).

(19) The work carried out by G. B. on this investigation constitutes a portion of the requirements for the Ph.D. degree.

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