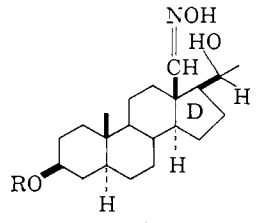
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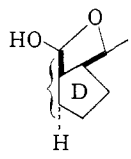
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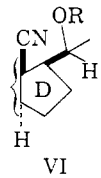
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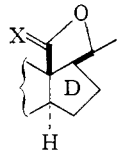
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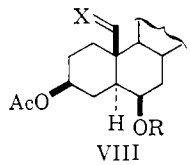
VIII

oxime, m.p. (from methanol) 184–185°, $[\alpha]_D$ −17° (*c*, 0.71) (15.2 g.). The structure of the oxime was proved by a series of transformations analogous to those described above for the first example of the reaction.

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M. M. PECHET

RECEIVED APRIL 7, 1960

A SYNTHESIS OF ALDOSTERONE ACETATE

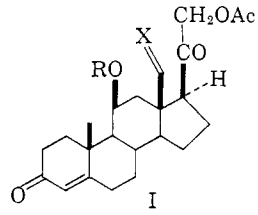
Sir:

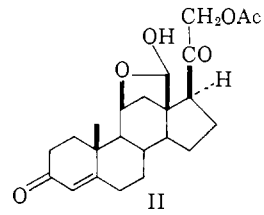
We wish to report a convenient three step partial synthesis of aldosterone acetate[1] using the new photochemical reaction reported in the preceding communication.[2] Corticosterone acetate (I, R = H, X = H_2) in dry pyridine was treated with excess nitrosyl chloride at room temperature to give corticosterone acetate nitrite (I, R = NO, X = H_2), m.p. 174–176°, $[\alpha]_D$ +316° (*c*, 1.1; all rotations in $CHCl_3$). This nitrite (4.0 g.) in toluene (200 ml.) was irradiated at 32° under pure nitrogen for 75 min. as described.[2] The crystalline solid which had separated was removed (885 mg., 21.2%) and identified as aldosterone acetate oxime (I, R = H, X = NOH). Recrystallized from benzene this had m.p. 175–194°, $[\alpha]_D$ +198° (*c*, 1.3), λ_{max} 240 mμ (ε = 16,500), $\nu_{max}^{CHCl_3}$ 3550, 3350, 1740, 1665 and 1615 cm.$^{-1}$. Treatment with pyridine–acetic anhydride at 100° for 5 min. gave the oxime acetate (I, R = N, X = NOAc), m.p. (from ethyl acetate) 183–187°, $\nu_{max}^{CHCl_3}$ 3650, 1780, 1750, 1675 and 1625 cm.$^{-1}$. Aldosterone acetate oxime (505 mg.) was added at 10° to a mixture of acetic acid (8 ml.) and aqueous sodium nitrite (5%, 4 ml.) and kept with agitation for 5 min. Extraction with methylene chloride gave, on crystallization from ethyl acetate, aldosterone 21-acetate (II) (320 mg.), identified by m.p., rotation, analysis, ultraviolet and infrared spectra (comparison with authentic racemate) and by paper chromatography.

On melting, or on refluxing in methanol for 1 hr., aldosterone acetate oxime was converted into the nitrone (III), m.p. 194–197°, $[\alpha]_D$ +119° (*c*, 1.0), λ_{max} 239 mμ (ε = 27,400), ν_{max}^{KBr} 1735, 1660 and 1600 cm.$^{-1}$. The usual infrared band for a 20-ketone was absent. We shall discuss the mechanism of the nitrite photolyses reported here and in the preceding communication[2] in our complete paper.

Very recently an alternative partial synthesis of aldosterone has been reported involving about 20 steps from 3α-acetoxypregnane-11,20-dione.[3]

It is a pleasure to acknowledge the encouragement and help that we have at all times received from Dr. M. M. Pechet. Skillful technical assistance was provided by Mrs. M. A. Golub, Misses R. A. Holland and M. A. Kennedy and by Mr. P. C. Ludwig for the work described in this and in the preceding Communication.


I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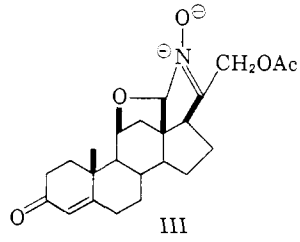
III

(1) S. A. Simpson, T. F. Tait, A. Wettstein, R. Neher, J. V. Euw, O. Schindler and T. Reichstein, *Helv. Chim. Acta*, **37**, 1163, 1200 (1954).

(2) D. H. R. Barton, J. M. Beaton, L. E. Geller and M. M. Pechet, THIS JOURNAL, **82**, 2640 (1960).

(3) K. Heusler, J. Kalvoda, C. Meystre, P. Wieland, G. Anner, A. Wettstein, G. Cainelli, D. Arigoni and O. Jeger, *Experientia*, **16**, 21 (1960). See also L. Velluz, G. Muller, R. Bardoneschi and A. Poittevin, *Compt. rend.*, 725 (1960).

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RECEIVED APRIL 7, 1960

STRUCTURE OF O,O'-DIETHYL METHYLPHOSPHONOTHIOATE AND CONJUGATIVE PROPERTIES OF THE P=S BOND

Sir:

In connection with more comprehensive studies on the nature of heteroörganic bonds, we have examined recently the structure of O,O'-diethyl methylphosphonothioate, $CH_3P(S)(OCH_2CH_3)_2$, and have ascertained a novel structural feature which has important implications for reaction mechanisms involving this and analogous organic phosphorus systems. Employing high-resolution nuclear resonance equipment,[1] we have obtained fine details of both the H^1 and P^{31} spin-resonance transitions of this compound in an extremely homogeneous magnetic field of 14,092 gauss. Two fixed-frequency oscillators were used successively to irradiate the sample. The material was especially purified for this and other studies.

In the H^1 trace, the two expected higher-field band patterns stand out clearly: an equal inten-

(1) Varian Associates V4300-2.

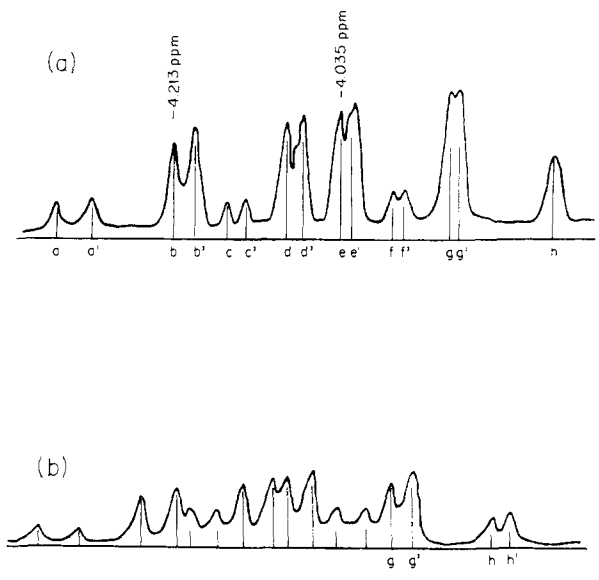

Fig. 1.—(a). H^1 magnetic resonance spectrum of the methylenic band in undiluted 0,0′-diethyl methylphosphonothioate. Corresponding lines from each of the two overlapping multiplets are indicated.

(b). Same as (a), but compound diluted to 35 volume % in benzene. The lines more adequately resolved by this technique are indicated.

multiplets were partially overlapping in the methylene region, a most unusual circumstance in the light of the usually assigned structure for this compound.

Although the final purification procedure used to prepare the sample indicated no excessive contamination, further corroboration of the sample purity and structure appeared necessary in view of the unusual proton spectrum. Accordingly, the P^{31} spectrum was examined at 24.288 mc./sec. A single chemical shift showing considerable fine structure was measured at −94.9 ppm. (referenced externally from 85% H_3PO_4). This, in conjunction with the H^1 trace, indicated that at least 99% of the sample consisted of a single type of phosphorus ester and that the general structure of the compound was of the phosphonothionate type.[3] The P^{31} fine structure was then studied and an analysis of the pattern was made. The methyl of the ethoxy group shows negligible spin coupling with the phosphorus nucleus,[4] simplifying the calculation to that for a model involving two sets of non-equivalent protons coupled to a single phosphorus nucleus. On the plausible assumption[5] that, in this situation, no mixing occurs between the spin state basis product functions of any one set with those of another, a symmetrical triangular nine-line pattern was calculated. The observed

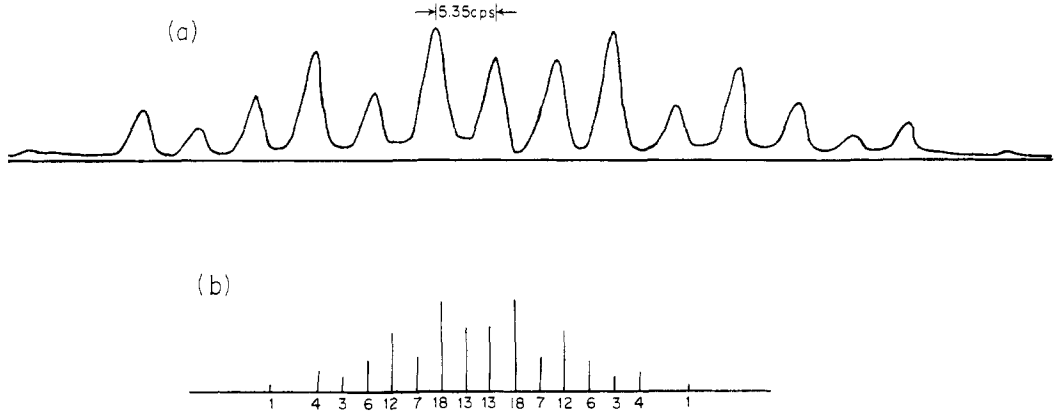

Fig. 2.—(a) Fine structure in the experimentally obtained P^{31} magnetic resonance spectrum of 0,0′-diethyl methylphosphonothioate. (b) Theoretical P^{31} spectrum on the assumption of two sets of non-equivalent methylenic protons in the molecule. Theoretical line intensities are indicated.

sity doublet (J_{CH_3-P} = 15.5 cps., δ = −1.723 ppm., referenced internally from Me_4Si), due to the energy level transitions of the protons of the methyl group directly bonded to the P atom, and a 1:2:1 triplet (J_{CH_3-C} = 6.6 cps., δ = −1.260 ppm.) due to the transitions of the non-equivalent protons in the methyl bonded to the methylene group. An entirely unexpected band pattern, however, developed in the low field position assigned to the methylene proton transitions (Fig. 1a).

The band pattern is quite unlike that predicted on the basis of the perturbation treatment developed for calculating the energy levels and transition probabilities for three sets of coupled nuclei.[2] It appeared probable, therefore, that two or more spectrum (Fig. 2a) shows no correspondence with this pattern, and it was at once clear from the P^{31} spectrum that more than two non-equivalent sets of protons were involved.

A calculation then was made of the expected transition probabilities and frequencies for a model based on two sets of non-equivalent methylenic protons as well as the single set of methyl (bonded to P) protons, with all three sets spin-coupled to P. The predicted spectrum of sixteen lines, both as regards relative line intensities and frequency distribution, was found to correspond

(2) W. A. Anderson, *Phys. Rev.*, **102**, 151 (1956).

(3) H. Finegold, *Ann. N. Y. Acad. Sci.*, **70**, 875 (1958).

(4) H. Finegold, to be published.

(5) J. A. Pople, W. G. Schneider and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, pp. 103–161.

precisely to the experimentally obtained trace (Fig. 2a,b).

While the P^{31} multiplet pattern clearly pointed to the non-equivalence of the methylenic protons, final corroboration was effected by returning to the proton resonance pattern. The coupling constant (10.7 cps.) obtained from the P^{31} trace was found to correspond with acceptable precision to the mean of the experimentally measured coupling constants from the H^1 methylenic trace, on the assumption of two similar, but chemical-shifted, methylenic multiplets with slightly different coupling constants (J_{P,CH_2} = 10.7 cps., $J_{P,C'H_2}$ = 10.3 cps.). The detailed pattern of each of the multiplets could not, however, be clearly established because of some overlap of fine structure. In order to obviate this difficulty, the magnetic anisotropy of benzene[6] was utilized in an ancillary fashion to increase the chemical shift separation of the two overlapping multiplets.

Several solutions of the phosphothionate ester of varying concentration in benzene were prepared. As expected, the difference in relative shieldings of the non-equivalent methylenic protons showed a concentration dependence, and degeneracies were removed at a sample concentration of 35 volume % in benzene solution (Fig. 1b). The emergent pattern became that of two similar multiplets, the fine structure of each corresponding to the predicted second order perturbation treatment of the energy levels for this type of system.

The non-equivalence of the two ethoxy groups in the molecule therefore has been unequivocally established by both the H^1 and P^{31} spectra. In order better to evaluate the role of restricted internal rotation in the phenomenon, studies of the temperature dependence of the spectrum were conducted with the induction probe as modified by a variable temperature sample cavity. The critical n.m.r. parameters remained invariant between 300–500°K., indicating a rather striking stability of structure associated with the non-equivalent methylenic groups. This can be explained in terms of preferred ethoxy group orientations about P–O(R) single bonds. However, on the basis of chemical reactivity, the author prefers, and proposes to elaborate elsewhere, an explanation based on unequal P–O(R) bond orders in some molecular systems containing p,d pi bonds. The net effect can be likened to a resonance stabilization of the molecule in which the anti-bonding electrons of only one of the oxygen atoms contribute to canonical structures involving a conjugated P=S bond.

A final feature may be noted. The molecular asymmetry as demonstrated by the nuclear resonance evidence can lead to the prediction of possible optical activity for this compound.

The author thanks R. Schumm and E. J. Prosen for their coöperation in making available the purified sample used in this study.

NATIONAL BUREAU OF STANDARDS
CHEMISTRY DIVISION HAROLD FINEGOLD
WASHINGTON 25, D. C.

RECEIVED MARCH 7, 1960

(6) A. A. Bothner-By and R. E. Glick, *J. Chem. Phys.*, **26**, 1651 (1957).

ON THE MECHANISM OF THE COBAMIDE COENZYME DEPENDENT ISOMERIZATION OF METHYLMALONYL CoA TO SUCCINYL CoA

Sir:

It has been established that the isomerization of methylmalonyl CoA[1] to succinyl CoA is a key step in the metabolism of propionate[2] and that a cobamide coenzyme[3] is an obligatory co-catalyst for this isomerization.[4,5] The reaction mechanism now has been investigated using 2-C^{14}-methylmalonyl CoA.[6] With this substance, migration of the carboxyl group to the beta methyl carbon would yield 2-C^{14}-succinyl CoA, whereas movement of the thiolester group would yield 3-C^{14}-succinyl CoA.

$$\begin{array}{l} HO_2CCH_2\overset{*}{C}H_2COSCoA \\ \quad\uparrow \text{ } CO_2H \text{ migration} \\ \qquad CH_3 \\ \qquad | \\ HO_2C\overset{*}{C}HCOSCoA \\ \quad\downarrow \text{ COSCoA migration} \\ HO_2C\overset{*}{C}H_2CH_2COSCoA \end{array}$$

In the experiment described in Table I, the 2-C^{14}-methylmalonyl CoA was isomerized by incubation with an enzyme preparation from *Propionibacterium shermanii*. The acyl-CoA compounds thus formed were converted to their acid amide derivatives by treatment with concentrated ammonium hydroxide and, after the addition of 550 mg. of carrier succinic acid amide, the C^{14}-succinic acid amide was crystallized to constant specific activity[7] and was degraded to determine the distribution of the isotope. To carry out the degradation (1) the succinic acid amide was converted to β-alanine and carbon dioxide by a Hofmann degradation.[8] The carbon dioxide is derived from carbon 1 of the succinyl CoA. (2) The β-alanine was converted to acrylic acid by exhaustive methylation with dimethyl sulfate.[9] The acrylic acid was reduced to propionic acid which was degraded stepwise to carbon dioxide and methylamine by the Schmidt reaction as modified by Phares.[10] Carbons 1, 2, and 3 of the propionic acid thus correspond to carbons 4, 3 and 2, respectively, of the succinyl CoA. The data summarized in Table I show that 80% of the isotope was in carbon 3 of succinyl CoA.[11] It is thus

(1) Coenzyme A is abbreviated as CoA.

(2) M. Flavin and S. Ochoa, *J. Biol. Chem.*, **229**, 965 (1957); R. E. Swick and H. G. Wood, *Proc. Natl. Acad. Sci. U. S.*, **46**, 28 (1960).

(3) H. Weissbach, J. Toohey and H. A. Barker, *ibid.*, **45**, 521 (1959).

(4) E. R. Stadtman, P. Overath, H. Eggerer and F. Lynen, *Biochem. Biophys. Research Comm.*, **2**, 1 (1960).

(5) J. R. Stern and D. L. Friedman, *ibid.*, **2**, 82 (1960).

(6) The 2-C^{14}-methylmalonyl-CoA was prepared by the enzyme catalyzed transcarboxylation reaction: methylmalonyl CoA + 2-C^{14}-propionyl CoA ⇆ 2-C^{14}-methylmalonyl CoA + propionyl CoA (4).

(7) The succinic acid amide contained no 2-C^{14}-methylmalonic acid amide.

(8) "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, New York, N. Y., 1943, p. 19.

(9) R. Willstätter, *Chem. Ber.*, **35**, 584 (1902).

(10) E. F. Phares, *Archiv. Biochem. Biophys.*, **33**, 173 (1951).

(11) The small amount of isotope found in the number 2 carbon atom probably is due to the enzyme (CoA-transphorase) catalyzed equilibration of 3-C^{14}-succinyl CoA with its symmetrical hydrolysis product, 2,3-C^{14}-succinate, which is produced in trace amounts under the experimental conditions. Evidence that such equilibration can account