The movement of many DNP-peptides with 3AA-15A-B is more rapid than is generally desirable. The use of 2AA-10A-B or 1AA-5A-B accordingly is indicated. The usefulness of this type of developer lies in its ability in some instances to invert the relative positions of certain DNP-derivatives.

Applications

We have made extensive use of the above information in the study of peptides from partial hydrolysates of gelatin.[4] The chromatographic behavior of all of the peptides which have been identified has agreed very well with that which would be expected from the generalizations which have been formulated above.

In attempting to separate a mixture of unknown DNP-peptides, it has been found to be beneficial to develop first with 7 *V* ml. of 8AA-4A-L. In this way, any contaminating DNP-amino acids will largely be removed and any weakly adsorbed DNP-peptides will begin to move down the chromatogram. Further development with as much as 7 *V* ml. of 3AA-15A-L will often separate the DNP-peptides into well-defined well-separated zones but depending upon the nature of the peptides it may be necessary to change further to 4AA-20A-L or 5AA-25A-L. Each zone should then be rechromatographed with 1AA-5A-B or stronger developer of the same type. By the use of this procedure it was possible to separate 14 definite DNP-derivatives from one peptide zone which was isolated from a partial hydrolysate of gelatin by means of an initial ion exchange chromatogram of the free peptides.

Acknowledgment.—We wish to thank Miss Lois M. Kay and Dr. F. Charlotte Green for having determined the chromatographic properties of some of the DNP-peptides.

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Ethyl Trithioörthoglyoxylate

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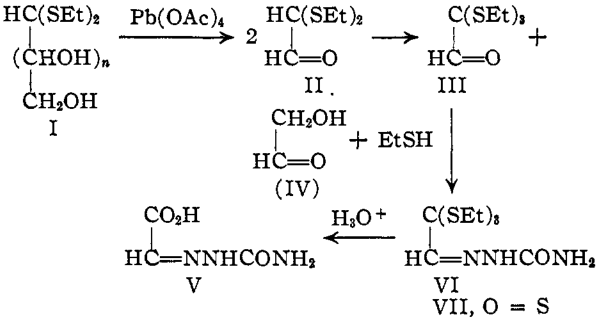
Fischer and Baer[1] synthesized the mono-(diethyl acetal) of glyoxal by subjecting DL-glyceraldehyde diethyl acetal to lead tetraacetate oxidation. The compound was a liquid that polymerized on standing. Schmidt and Wernicke[2] prepared glyoxal mono-(dibenzyl thioacetal) as a crystalline solid by the oxidative scission of 4,5-isopropylidene-D-fucose dibenzyl thioacetal with lead tetraacetate. In attempts to obtain the diethyl analog of this substance by the corresponding scission of aldose (D-galactose, L-arabinose and glyceraldehyde) diethyl thioacetals, the only product isolated (as the semicarbazone VI or thiosemicarbazone VII) was ethyl trithioörthoglyoxylate. Its structure was proved by the partial acid hydrolysis of its semicarbazone to the known glyoxylic acid semicarbazone (V). It is probable that the ethyl trithioorthoglyoxylate (III) arose as a bimolecular disproportionation product of the intermediate glyoxal mono-(diethyl thioacetal) (II). Fischer and Baer[1] demonstrated that two moles of glyoxal mono-(diethyl acetal), on treatment with alkali, underwent such a disproportionation to produce one mole of acid; the presence of glyoxylic acid and of glycolaldehyde were demonstrated in the acidified reaction mixture by these workers. The appearance of the thioörthoester in the glyoxylic acid moiety obtained by us is unexpected.



Experimental

Oxidative Scission of Aldose Thioacetals.—An amount of 2.86 g. (0.01 mole) of D-galactose diethyl thioacetal (mercaptal)[3,4] was dissolved in 500 ml. of dry dioxane in an apparatus protected from moisture. Lead tetraacetate (17.6 g., 0.04 mole) was added portionwise under stirring while maintaining the reaction mixture at 10–20°. After the addition, the cooling bath was removed and stirring was maintained for 2 hr. Lead diacetate was separated by filtration and the solvent was removed, under reduced pressure, from the filtrate. The reaction product was recovered by distillation under reduced pressure, yield 1.1 g., b.p. 100° (1 mm.).

From the product there were isolated, in low yield, the crystalline semicarbazone and thiosemicarbazone described below. These were also obtained: from the reaction mixture before distillation; on similar oxidation in benzene solution or in abs. chloroform–acetic acid solution; and on oxidation of the diethyl thioacetals (mercaptals) of L-arabinose and of glyceraldehyde.[5]

Ethyl Trithioörthoglyoxylate Thiosemicarbazone (VII).—Following Wolfrom and Tanghe,[6] the above distilled oil was dissolved in 100% ethanol, and thiosemicarbazide was dissolved in a 30% solution of acetic acid in ethanol. The two solutions were heated just short of boiling and then mixed. After cooling to room temperature, water was added to incipient turbidity and crystallization was effected at ice-box temperature; m.p. 140.5–141°.

Anal. Calcd. for $C_9H_{19}N_3S_4$: C, 36.33; H, 6.44; N, 14.13; S, 43.10. Found: C, 36.47; H, 6.37; N, 14.14; S, 43.26.

Ethyl Trithioörthoglyoxylate Semicarbazone (VI).—Semicarbazide hydrochloride (1 g.) was dissolved in 10 ml. of *N* potassium hydroxide and then more solid semicarbazide hydrochloride was added until the solution was just acid. The above distilled oxidation product (1 g.), dissolved in 10 ml. of 95% ethanol, was warmed to 90° and the semicarbazide solution was added to it. More water was added to effect complete solution at that temperature and a crystalline product separated on slow cooling. Pure material was obtained on recrystallization from 95% ethanol; m.p. 195°.

Anal. Calcd. for $C_9H_{19}ON_3S_3$: C, 38.41; H, 6.81; N, 14.94; S, 34.18. Found: C, 38.63; H, 6.84; N, 14.92; S, 33.89.

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[1] H. O. L. Fischer and E. Baer, *Helv. Chim. Acta*, **18**, 514 (1935).

[2] O. T. Schmidt and E. Wernicke, *Ann.*, **556**, 179 (1944).

[3] E. Fischer, *Ber.*, **27**, 673 (1894).

[4] M. L. Wolfrom, This Journal, **52**, 2466 (1930).

[5] H. W. Arnold and W. L. Evans, *ibid.*, **58**, 1950 (1936).

[6] M. L. Wolfrom and L. J. Tanghe, *ibid.*, **59**, 1601 (1937).

Hydrolysis of Ethyl Trithioörthoglyoxylate Semicarbazone to Glyoxylic Acid Semicarbazone (V).—The above ethyl trithioörthoglyoxylate (400 mg.) was added to a mixture of 66 ml. of ethanol and 10 ml. of 12 *N* (concd.) hydrochloric acid. A stream of nitrogen gas was passed through the mixture while it was refluxed under a condenser containing water at 40°. The reaction was stopped when no more ethanethiol was evolved as detected by odor. Solvent removal under reduced pressure yielded a product that was obtained crystalline from 50% aqueous ethanol; m.p. 220–224° dec. An authentic sample of glyoxylic acid semicarbazone showed no melting point depression on admixture with the above product and the following X-ray powder diffraction measurements were identical when made on the two preparations: 6.48,[7] 1[8]; 4.53, 3; 4.25, 7; 3.58, 2; 3.13, 1; 2.81, 6; 2.46, 4; 2.26, 9; 1.89, 10.

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(7) Interplanar spacing in Å., CuKα radiation.

(8) Relative intensity; 1 = highest; estimated visually.

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Di-*t*-butyl Phosphonate

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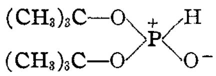
The reaction of *t*-butyl alcohol with phosphorus trichloride has been reported to give tri-*t*-butyl phosphite (I).[1] Previous to this publication, several attempts were made in this Laboratory to prepare I by analogous procedures, but instead di-*t*-butyl phosphonate (II) was obtained; b.p. 62–62.5° at 4 mm.,[2]


(II)

n^{25}D 1.4168, d^{25} 0.975; *M*D calcd. 50.18,[3] found 50.03. This appears to be the first example of the preparation of a tertiary dialkyl phosphonate from an aliphatic tertiary alcohol.[4]

II is insoluble in water and 3 *N* sodium hydroxide, but it dissolves immediately in 3 *N* hydrochloric acid with the evolution of isobutylene. This gas is also evolved slowly when the compound is heated to 70° at atmospheric pressure. II is relatively stable at room temperature, although evidence of slight decomposition was noted after storage for several weeks. Upon redistillation, the original material was recovered with little loss.

The infrared spectrum exhibits the characteristic strong absorption for the $\overset{+}{P}-\overset{-}{O}$ stretching vibration[5] at 1270 cm.$^{-1}$ as well as another strong band at 2420 cm.$^{-1}$ corresponding to the P–H bond.[5,6] There is little or no absorption in the 750 cm.$^{-1}$ region where the aliphatic C–P stretching frequency usually appears,[6] an observation which rules out structures which are based on catalytic isomerization of the Arbusov type.[7]

Although repetition of the literature procedure[1] afforded a substance having the reported refractive index, it could not be redistilled without decomposition and concomitant lowering of the refractive index. The infrared spectra showed strong P–H and $\overset{+}{P}-\overset{-}{O}$ absorption and were not significantly different from the spectrum of pure II. Successive redistillations eventually yielded material having a refractive index and infrared spectrum identical to II. In view of these results, the purity of I is doubtful.

Experimental[8]

A solution of 82.4 g. (0.6 mole) of phosphorus trichloride in 500 cc. of petroleum ether (b.p. 30–60°) was cautiously dropped over a period of one hour into an ice-methanol chilled solution of 267 g. (3.6 moles) of *t*-butyl alcohol and 91.2 g. (1.8 moles) of triethylamine in 3 l. of petroleum ether. Stirring was continued for one hour without external cooling, after which time the suspension was filtered, the cake being washed well with an additional 1 l. of petroleum ether. The solvent was removed on the steam-bath at water aspirator pressure, the resultant yellow residue being rapidly distilled at 0.1 mm. without fractionation. The distillate was distilled slowly (considerable frothing), giving 60 g. (51%), b.p. 72–78° at 10–12 mm., n^{25}D 1.4162. A final distillation gave a single fraction b.p. 70–72° at 10 mm., n^{25}D 1.4168, d^{25} 0.975.

Anal. Calcd. for $C_8H_{19}O_3P$: C, 49.5; H, 9.86; P, 15.9. Found: C, 49.5; H, 9.60; P, 15.8.

A portion of this product was redistilled at 4 mm., b.p. 62–62.5°. In various other preparations, samples of higher refractive index were obtained, but repeated slow distillations at 10–12 mm. eventually produced II of constant refractive index. The high refractive indices observed in some cases are probably due to the presence of some I which is decomposed on successive distillations.

Acknowledgment.—We are indebted to Mrs. Martha M. Taylor for the determination of the infrared spectra.

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(1) G. M. Kosolapoff, THIS JOURNAL, **74**, 4953 (1952).

(2) Compare b.p. 65–66° at 4 mm. for I (ref. 1). This b.p. was obtained by rapid distillation without fractionation (G. M. Kosolapoff, private communication).

(3) The value of 4.44 for the atomic refraction of phosphorus in secondary phosphites was used; G. M. Kosolapoff, *ibid.*, **73**, 4989 (1951).

(4) T. Milobedzki and A. Sachnowski, (*Chem. Polski*, **15**, 34 (1917) [*C. A.*, **13**, 2865 (1919)], report both tri- and di-*t*-butyl phosphites. They did not isolate either compound or report any physical properties.

(5) N. B. Colthup, *J. Opt. Soc.*, **40**, 397 (1950).

(6) It is noteworthy that di-*n*-butyl phosphonate has two corresponding bands at 1265 and 2410 cm.$^{-1}$; L. W. Daasch and D. C. Smith, *Anal. Chem.*, **23**, 853 (1951).

(7) T. Milobedzki and K. Szulgin, *Chem. Polski*, **15**, 66 (1917), [*C. A.*, **13**, 2866 (1919)], and numerous references given by G. M. Kosolapoff, "Organophosphorus Compounds," John Wiley and Sons, Inc., New York, N. Y., 1951.

(8) (a) The analysis was performed in these laboratories under the direction of Dr. J. A. Kuck. (b) All operations were performed under an atmosphere of dry nitrogen.