water was added to the dark red solution. Acidification with 50 ml. of 0.5 *N* HCl gave a fine crystalline, colorless precipitate, m.p. 155°, yield 2 g. (75%). Recrystallization from warm chloroform raised the melting point to 163–165°. The substance turned yellow upon melting.

Anal. Calcd. for $C_{16}H_{12}O_4$ (268.26): C, 71.63; H, 4.51. Found: C, 71.41; H, 4.37.

3-Phthalanylidenephthalide (V).—Phthalide (2.68 g., 20 mmoles) in 10 ml. of dimethyl sulfoxide was added to a solution of 2.16 g. of sodium methoxide (40 mmoles) in 35 ml. of dimethyl sulfoxide at 60–65°. The solution turned orange, and after a few minutes brown-red. The reaction mixture was kept at 65° for 25 min. Then about half of the solvent was removed by distillation at about 1 mm. and 65° bath temperature. Addition of 25 ml. of ice-water and 20 ml. of concentrated hydrochloric acid gave a cloudy solution that was twice extracted with 50 ml. of chloroform. The organic layer was separated and the solvent was removed by distillation. To remove traces of water and dimethyl sulfoxide, the oily residue was subjected to vacuum distillation at 1 mm. and 70° bath temperature. Treatment of the yellow, oily residue with methanol gave yellow crystals that exhibit a greenish fluorescence, m.p. 225–227°, yield 1.06 g. (42%). The substance can be recrystallized from hot chloroform. It was only slightly soluble in methanol.

Anal. Calcd. for $C_{16}H_{10}O_3$ (250.24): C, 76.79; H, 4.03. Found: C, 76.90; H, 4.18; mol. wt., 287 (thermoelectric measurement in dioxane).

V from IV.—IV, 250 mg., was placed in a test tube and heated for 15 min. at 185°. The colorless substance turned yellow with evolution of gas. The solid yellow residue was washed with a mixture of chloroform and methanol, yielding 210 mg. of V (90%), m.p. 225–227°; mixture melting point with V obtained by direct self-condensation of phthalide showed no depression.

***cis*-Biphthalide** (VII).—Potassium *t*-butoxide (2.4 g., 21 mmoles) was added to a solution of 3.3 g. of phthalaldehydic acid pseudo methyl ester (3-methoxyphthalide, 20 mmoles) in 50 ml. of dimethylformamide. The deep red solution was kept under nitrogen for 20 min. Addition of 50 ml. of ice-water and slow addition of 100 ml. of 0.5 *N* HCl gave a colorless precipitate (needles) in 400-mg. yield (15%). The substance can be recrystallized from boiling acetic acid; m.p. 334–335°. It exhibited a bright, blue fluorescence. (*trans*-Biphthalide fluoresces yellow.)

Anal. Calcd. for $C_{16}H_8O_4$(264.22): C, 72.73; H, 3.05. Found: C, 72.84; H, 3.08; mol. wt. 286 (osmometric in benzene).

Extraction of the aqueous filtrate with chloroform yielded 250 mg. of yellow needles besides an unidentifiable resin. The infrared spectrum of the yellow crystalline material indicated a mixture of *cis*- and *trans*-biphthalide.

Conversion of *trans*-Biphthalide into *cis*-Biphthalide.—*trans*-Biphthalide (264 mg., 1 mmole) was dissolved in 30 ml. of concentrated sulfuric acid at 110° and kept at this temperature under nitrogen for 1 hr. Addition of about 80 g. of ice gave an almost colorless precipitate. It was recrystallized from hot acetic acid, giving 240 mg. of very faintly yellow needles, m.p. 320°. According to ultraviolet and infrared spectra the product was *cis*-biphthalide contaminated with about 20% *trans*-biphthalide.

Dianhydrophenylosazone Acetates from Isomaltose and Gentiobiose

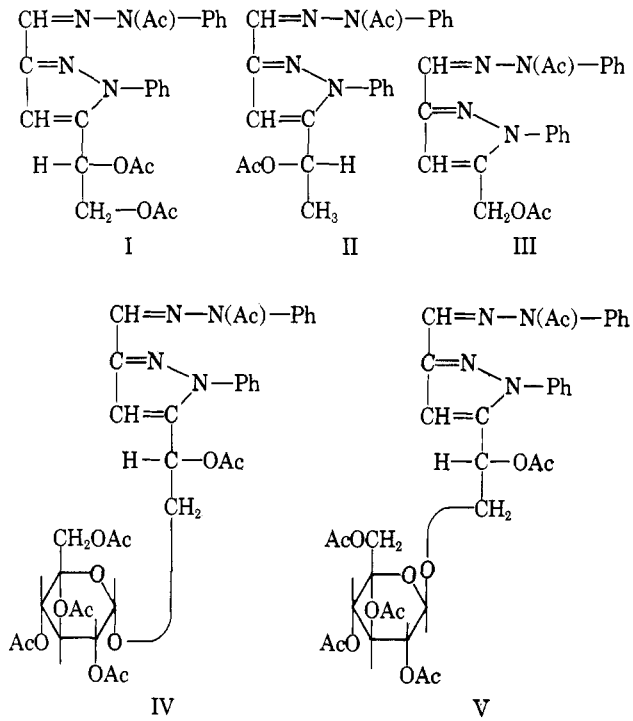
H. EL KHADEM[1]

Department of Chemistry, The Ohio State University, Columbus, Ohio 43210

Received April 3, 1964

Boiling acetic anhydride converts[2,3] monosaccharide phenylosazones into dianhydrophenylosazone acetates which have a pyrazole ring structure. The structure I was established for the product from any D-hexose precursor by transhydrazonation experiments and, after *O*-deacetylation, by oxidation to give known pyrazole derivatives. Similarly, the dianhydrophenylosazone acetates from the 6-deoxy-L-hexoses and the pentoses were assigned the structures II and III, respectively.

This work describes the unambiguous characterization of I, II, and III by n.m.r. spectroscopy and also reports the conversion of the disaccharides isomaltose and gentiobiose into the corresponding dianhydrophenylosazone acetates (IV and V, respectively).



The n.m.r. spectra of the compounds I, II, and III (Fig. 1–3) permit detailed structural analysis. Aromatic resonances in the τ 2.5–2.7 region may be assigned to the phenyl group protons and the proton of the pyrazole ring. The singlet of unit-proton intensity at τ 3.08, 2.98, and 3.05 in the three spectra respectively may be assigned to the strongly deshielded methine hydrogen of the hydrazone function. A singlet of three-proton intensity appears at τ 7.42, 7.40, and 7.44 in each spectrum, respectively, and may be assigned to the methyl protons of the *N*-acetyl group. The other singlet at τ 8.01, 8.10, and 7.89, respectively, may be assigned to the methyl protons of the *O*-acetyl groups; for II and III this peak has intensity of three protons, while for I the peak is of six-proton intensity. The spectrum of III shows a two-proton singlet, τ 4.90, attributable to the methylene group from C-5 of the pentose precursor. The methyl group of II, corresponding to C-6 of the 6-deoxy-L-hexose precursor, appears as a three-proton doublet, τ 8.45, being split by the adjacent proton, with a spin–spin coupling constant, J = 6.5 c.p.s. The latter proton, from C-5 of the 6-deoxy-L-hexose, appears as the expected quartet, τ 4.08, of unit-proton intensity, spin–spin coupling constant, J = 6.5 c.p.s. The D-hexose derivative I showed the methylene group from C-6 of the hexose as a two-proton doublet,

(1) Chemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt, U. A. R.

(2) H. El Khadem and M. M. Mohammed-Ali, *J. Chem. Soc.*, 4929 (1963).

(3) H. El Khadem, Z. M. El-Shafei, and M. M. Mohammed-Ali, *J. Org. Chem.*, **29**, 1565 (1964).

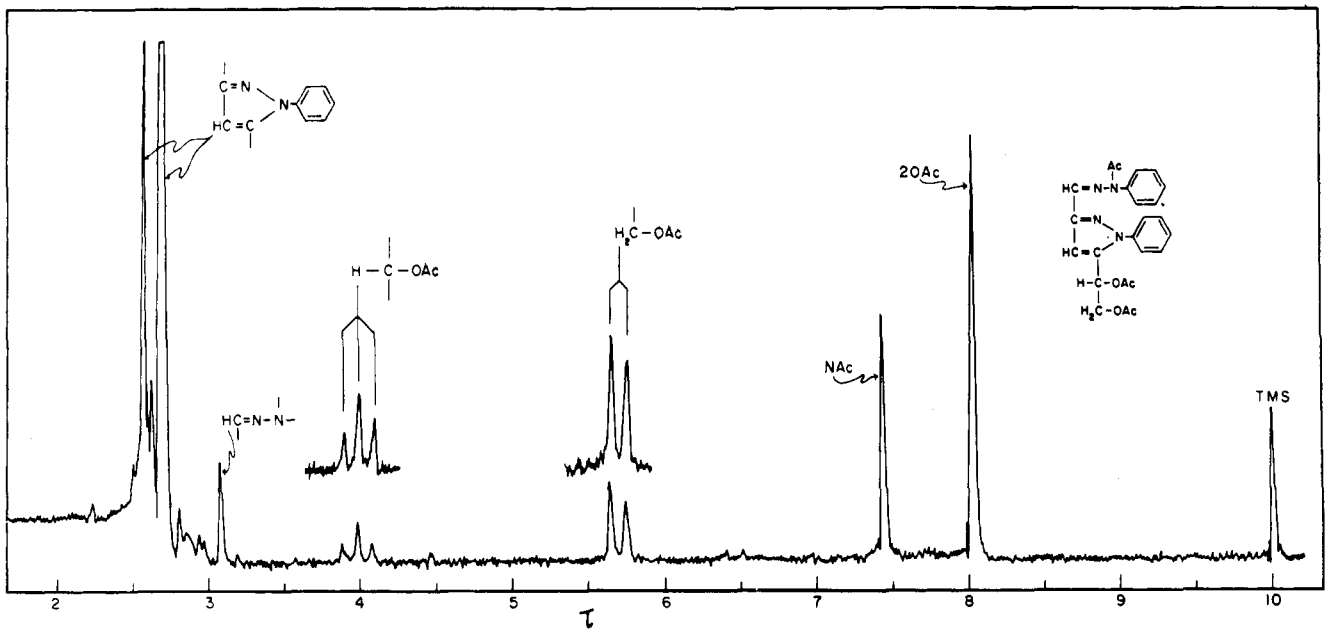


Fig. 1.—N.m.r. spectrum of 5-(D-*glycero*-1,2-diacetoxyethyl)-3-formyl-1-phenylpyrazole *N*-acetylphenylhydrazone (I).

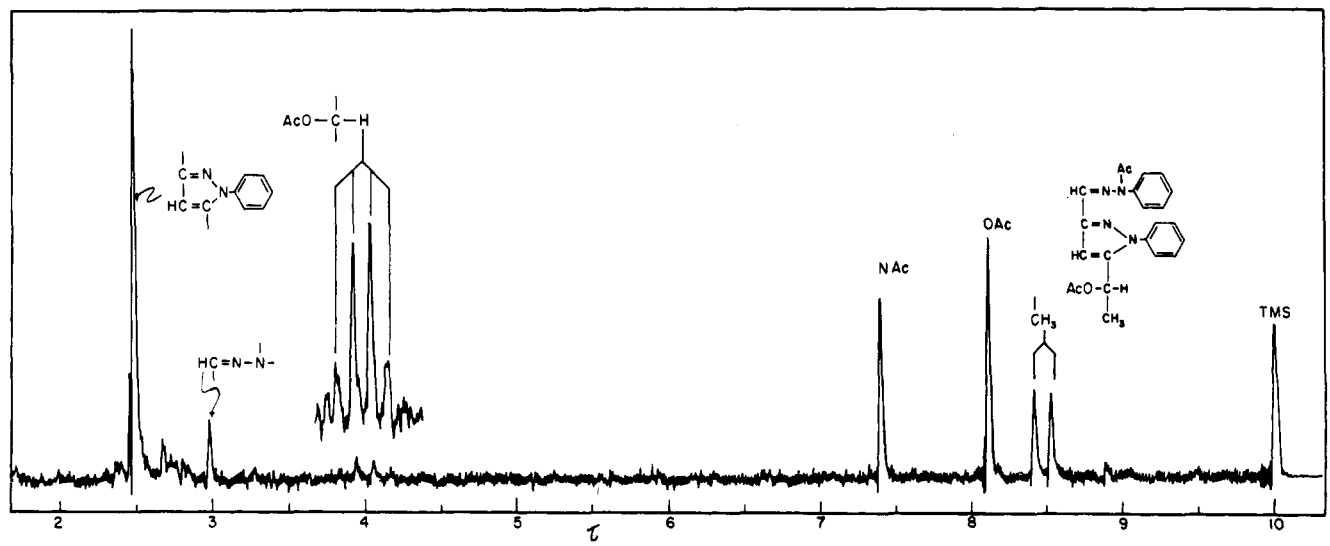


Fig. 2.—N.m.r. spectrum of 5-(L-*glycero*-1-acetoxyethyl)-3-formyl-1-phenylpyrazole *N*-acetylphenylhydrazone (II).

τ 5.70, split by the adjacent proton, J = 6.0 c.p.s. The latter proton, from C-5 of the hexose, gives the expected unit proton triplet at τ 3.95 with J = 6.0 c.p.s.

The dianhydrophenylosazone acetate structure (I–III) would be expected from reducing disaccharides possessing (1→5) or (1→6) links, the (1→2) link would prevent osazone formation and the (1→3) and (1→4) links would stop the formation of a pyrazole ring. Our findings indicate that the phenylosazones of α-D-glucopyranosyl-(1→3)-D-arabinose,[4] maltose, cellobiose, and lactose were indeed simply acetylated in the presence of refluxing acetic anhydride while the (1 → 6)-linked disaccharides, isomaltose and gentiobiose, were converted readily to the expected dianhydrophenylosazone acetates, IV and V, respectively.

Experimental[5]

Dianhydrophenylosazone Hexaacetate (IV) from Isomaltose Phenylosazone.—The osazone[6] (1.2 g.) was refluxed with acetic anhydride (50 ml.) for a period of 1 hr., then poured into crushed ice (0.5 kg.). The aqueous solution was decanted and the oily residue was washed twice with cold water, then crystallized by dissolving in hot 50% ethanol (25 ml.) and filtering the cooling solution upon turbidity. 2-(3-Formyl-1-phenylpyrazol-5-yl)-2*S*-2-acetoxyethyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside acetylphenylhydrazone crystallized in colorless needles: yield 1 g. (60%); m.p. 190°; $[\alpha]^{20}D$ +118° (*c* 0.55, chloroform); λ_{max}^{EtOH} 282 mμ (log ε 4.46); ν_{max}^{KBr} 1740 (O–Ac), 1690 (N–Ac), 1600 cm.$^{-1}$ (C=N); X-ray powder diffraction pattern[7]: 14.25 w, 10.78 s, 7.89 m, 5.06 w, 4.77 w, 4.46 w, 4.25 w, 4.09 m, 3.91 w, 3.60 w, 3.45 m, and 3.03 w.

(4) G. Zemplen, *Chem. Ber.*, **60**, 1555 (1927).

(5) Infrared spectra were measured with a Perkin-Elmer Infracord spectrophotometer; ultraviolet spectra were measured on a Bausch and Lomb Model 505 spectrophotometer; n.m.r. spectra were obtained in deuteriochloroform solutions on a Varian Associates apparatus, Model A60. Microanalytical determinations were made by W. N. Rond, The Ohio State University.

(6) A. Thompson and M. L. Wolfrom, *J. Am. Chem. Soc.*, **76**, 5173 (1954).

(7) Interplanar spacing, Å., Cu Kα radiation. Relative intensities estimated visually: s, strong; m, medium; w, weak; v, very.

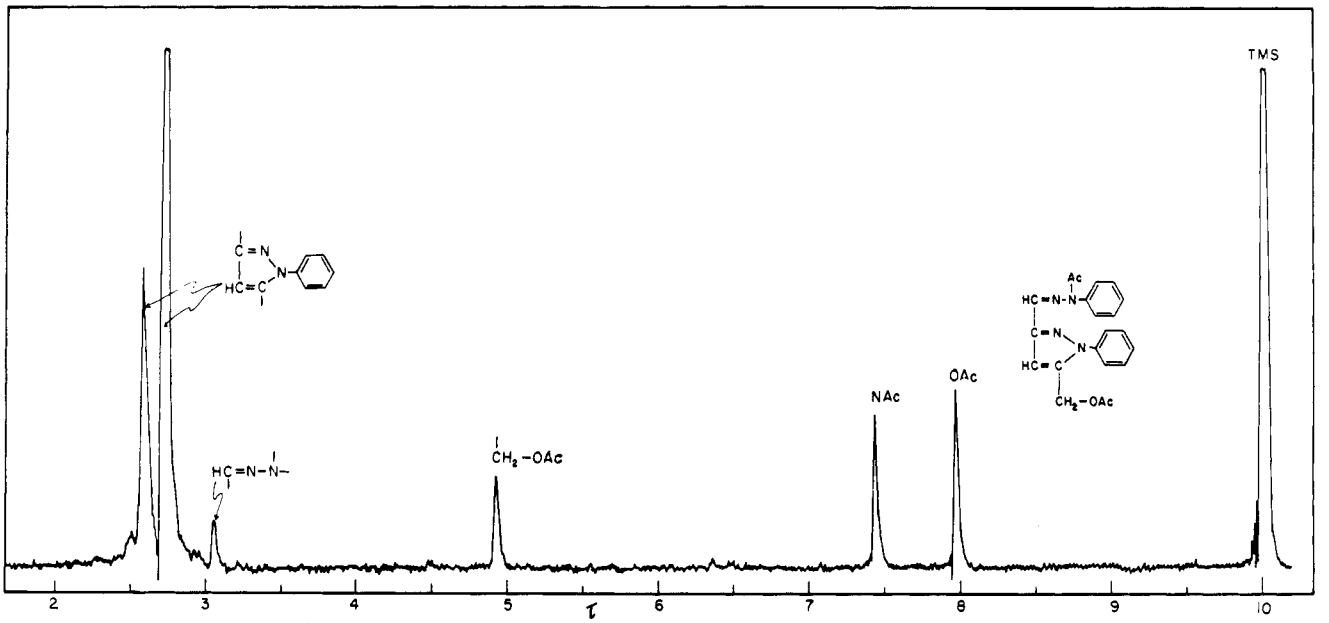


Fig. 3.—N.m.r. spectrum of 5-acetoxymethyl-3-formyl-1-phenylpyrazole *N*-acetylphenylhydrazone (III).

Anal. Calcd. for $C_{36}H_{40}N_4O_{13}$: C, 58.69; H, 5.47; N, 7.60; total Ac, 35.06; O-Ac, 29.21. Found: C, 59.19; H, 5.93; N, 8.17; total Ac, 34.18; OAc, 28.34.

Dianhydrophenylosazone Hexaacetate (III) from Gentiobiose Phenylosazone.—The osazone[8] (2.3 g.) was refluxed with acetic anhydride (50 ml.) for a period for 1 hr., then poured onto crushed ice (0.5 kg.). The aqueous solution was decanted and the oily residue crystallized on trituration with ethanol. 2-(3-Formyl-1-phenylpyrazol-5-yl)-2*S*-2-acetoxyethyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside acetylphenylhydrazone recrystallized from 50% aqueous ethanol in colorless needles: yield 2.2 g. (68%); m.p. 178°; $[\alpha]^{20}D$ +31.6° (*c* 0.475, chloroform); λ_{max}^{EtOH} 282 mμ (log ε 4.46); ν_{max}^{KBr} 1790 (O-Ac), 1685 (N-Ac), 1595 cm.$^{-1}$ (C=N); X-ray powder diffraction pattern[7]: 11.79 m, 10.78 s, 10.04 m, 7.76 s, 5.86 w, 5.40 w, 5.18 w, 4.82 vs, 4.17 m, 4.02 s, 3.86 m, 3.66 w, 3.48 m, and 3.23 m.

Anal. Calcd. for $C_{36}H_{40}N_4O_{13}$: C, 58.69; H, 5.47; N, 7.60; total Ac, 35.06; O-Ac, 29.21. Found: C, 58.63; H, 5.26; N, 8.01; total Ac, 34.38; O-Ac, 29.64.

Acknowledgment.—The author is indebted to the Educational and Cultural Exchange Program for a Fulbright Grant to visit the Ohio State University; to Professor M. L. Wolfrom, Dr. D. Horton, and Mr. F. Komitsky for their counsel; to The Ohio State University for the laboratory facilities provided; to Dr. A. Sato who kindly made available the isomaltose and gentiobiose used; to Dr. R. D. Nelson of Chemical Abstracts who helped in the nomenclature of the compounds.

(8) H. Berlin, *J. Am. Chem. Soc.*, **48**, 1107 (1926).

Amidino and Carbamoyl Osazones of Sugars

M. L. WOLFROM,[1] H. EL KHADEM, AND H. ALFES

Department of Chemistry, The Ohio State University, Columbus, Ohio 43210

Received May 29, 1964

1,2-Bis(amidinohydrazone) salts[2,3] are known[4] to be active against some forms of leukemia but have the disadvantage of producing toxic effects. It was considered that saccharide bis(amidinohydrazone) salts might be less toxic. These compounds can be considered as amidinoosazones but attempts to synthesize them according to the method used by Fischer[5] to prepare sugar osazones proved unsuccessful. We have therefore prepared them from aldosuloses ("osones") and 1-aminoguanidine sulfate. Thus, D-*arabino*-hexosulose bis(amidinohydrazone) monosulfate (Ia) was obtained from D-*arabino*-hexosulose ("D-glucosone"), L-*xylo*-hexosulose bis(amidinohydrazone) monosulfate (Ic) from L-*xylo*-hexosulose (from L-sorbose), and D-*threo*-pentosulose bis(amidinohydrazone) monosulfate (Id) from D-*threo*-pentosulose (from D-xylose). These compounds exist as slightly yellow crystals, soluble in hot water, from which they crystallize on cooling.

Although sugar disemicarbazones can be considered as carbamylosazones, they cannot be prepared by treating aldoses with semicarbazide as the reaction does not proceed beyond the semicarbazone stage; we have therefore prepared these from aldosuloses ("osones") and semicarbazide also. Thus, D-*arabino*-hexosulose disemicarbazone (IIa) was obtained from D-*arabino*-hexosulose ("D-glucosone"), D-*lyxo*-hexosulose disemicarbazone (IIb) from D-*lyxo*-hexosulose ("D-galactosone"), L-*xylo*-hexosulose disemicarbazone (IIc) from L-*xylo*-hexosulose ("L-gulosone"), and D-*threo*-pentosulose disemicarbazone (IId) from D-*threo*-pentosulose ("D-xylosone"). Unlike arylosazones these compounds were colorless and their ultraviolet spectra were characterized by a single maximum at 292 mμ instead of consecutive maxima stretching from 256 to 399 mμ in the case of the phenylosazones.[6] Furthermore, they possessed greater solubility in water than in organic solvents, excluding pyridine.

(1) To whom inquiries should be addressed.

(2) J. Thiele and E. Dralle, *Ann.*, **302**, 275 (1898).

(3) E. G. Podrebarac, W. H. Nyberg, F. A. French, and C. C. Cheng, *J. Med. Chem.*, **6**, 283 (1963); F. Baiocchi, *et al.*, *ibid.*, **6**, 431 (1963).

(4) B. L. Freedlander and F. A. French, *Cancer Res.*, **18**, 360, 1286 (1958).

(5) E. Fischer, *Ber.*, **17**, 579 (1884).

(6) V. C. Barry, J. E. McCormick, and P. W. D. Mitchell, *J. Chem. Soc.*, 222 (1955); G. Henseke and M. Winter, *Ber.*, **93**, 45 (1960).