volume of about 50 cc. Further concentration yielded two additional crops; total yield 13.4 g., m. p. 100–104°. Pure material was obtained on recrystallization from absolute ethanol (20 parts) by the addition of an equal volume of petroleum ether; m. p. 103–104°, spec. rot. +41° (24°, *c* 5, H_2O).[5]

The substance crystallized in plates and was soluble in water, methanol, ethanol and acetone, moderately so in ether, and was practically insoluble in petroleum ether.

Anal. Calcd. for $C_5H_{10}O_4(SC_2H_5)_2$: C, 42.16; H, 7.86; S, 25.01. Found: C, 42.05; H, 7.66; S, 24.8.

d-Lyxose Diethyl Mercaptal Tetraacetate.—*d*-Lyxose diethyl mercaptal (11.4 g.) was acetylated at room temperature (initial cooling) for twelve hours with pyridine (50 cc.) and acetic anhydride (100 cc.) and the crystalline product (18.5 g.) that separated on pouring the acetylation mixture into ice and water (one liter) was obtained pure from the minimum amount of ether by the addition of two volumes of petroleum ether; m. p. 36–37°, spec. rot. +40.5° (28°, *c* 5, abs. $CHCl_3$).

The substance crystallized in soft prisms and was soluble in the common solvents except water and petroleum ether.

Anal. Calcd. for $C_5H_6O_4(CH_3CO)_4(SC_2H_5)_2$: S, 15.10; CH_3CO, 9.42 cc. 0.1 *N* NaOH per 100 mg. Found: S, 14.83; CH_3CO, 9.46 cc.

This substance also was prepared directly from the sugar without the isolation of the mercaptal. *d*-Lyxose (13.5 g.) was mercaptalated as described previously and the hydrochloric acid was neutralized at 0° (addition of ice) by the cautious addition of concentrated ammonium hydroxide. The dried mixture of ammonium chloride and lyxose mercaptal obtained on solvent removal under reduced pressure was acetylated as described above and the product was isolated in the same manner; yield 23 g.

***aldehydo-d*-Lyxose Hexaacetate.**—Demercaptalation of *d*-lyxose diethyl mercaptal tetraacetate in moist acetone with mercuric chloride and cadmium carbonate according to the improved procedure of Wolfrom and Konigsberg[6] yielded only sirups that were not amenable to crystallization. An amount of 2 g. of such a sirup was acetylated for fifteen hours at room temperature (initial cooling) with pyridine (25 cc.) and acetic anhydride (50 cc.). The mixture obtained on pouring the brown solution into ice and water (300 cc.) was extracted with chloroform and the sirup obtained on solvent removal from the washed (successively with 5% sulfuric acid, aqueous sodium bicarbonate, and water) and dried extract was crystallized from methanol by the addition of water; yield 0.41 g. Pure material was obtained on further crystallization effected in the same manner; m. p. 87–88°, spec. rot. +13° (29°, *c* 3.6, U. S. P.[7] $CHCl_3$).

Anal. Calcd. for $C_5H_6O_6(CH_3CO)_6$: C, 48.57; H, 5.76; CH_3CO, 14.3 cc. 0.1 *N* NaOH per 100 mg. Found: C, 48.70; H, 5.64; CH_3CO, 14.2 cc.

This substance also was obtainable by acetolysis of the acetylated mercaptal according to the general procedure of Pirie.[4] *d*-Lyxose diethyl mercaptal tetraacetate (2 g.) was treated for eighteen hours at room temperature (initial cooling) with 35 cc. of acetic anhydride containing 1 cc. of concentrated sulfuric acid. The yellow sirup obtained on pouring the reaction mixture into ice and water (200 cc.) crystallized on standing overnight at ice-box temperature; yield 1.1 g. Pure material was obtained on purification from methanol–water; m. p. 87–88°, spec. rot. +13° (U. S. P. $CHCl_3$).

Summary

1. The synthesis is reported of three acyclic derivatives of *d*-lyxose: *d*-lyxose diethyl mercaptal, *d*-lyxose diethyl mercaptal tetraacetate, *aldehydo-d*-lyxose hexaacetate.

(5) All rotations are recorded to the D-line of sodium light; 24° is the temperature; *c* is the concentration in g. per 100 cc. soln.

(6) M. L. Wolfrom and M. Konigsberg, THIS JOURNAL, **61**, 574 (1939).

(7) United States Pharmacopoeia.

COLUMBUS, OHIO RECEIVED OCTOBER 17, 1940

[CONTRIBUTION FROM THE VEADER LEONARD LABORATORY OF EXPERIMENTAL THERAPEUTICS]

Alkyl Ethers of 2,4-Dinitrophenol as Stimulants of the Metabolic Rate

BY LAURENCE G. WESSON

The fatalities resulting from a rapid hyperthermia in isolated cases of dinitrophenol poisoning led us, in 1934, to attempt to develop derivatives of dinitrophenol that would have a more gradual and moderate action on the metabolism of the body. One such attempt that was apparently successful in so far as this particular phase is concerned, consisted in the preparation and testing of a series of new alkyl ethers of dinitrophenol. The ether that gave the greatest promise of usefulness was the isopropyl ether. This compound was characterized by a low degree of toxicity and a protracted increase of the metabolic rate under its influence, as well as favorable melting point and ease and cheapness of its preparation.

The first preparation of an alkyl ether of 2,4-dinitrophenol was that of Cahours[1] in 1850 who obtained the ethyl ether by the nitration of phenetol. In 1867, Gruner[2] used the alkyl iodide–

(1) A. Cahours, *Ann.*, **74**, 315 (1850).

(2) H. Gruner, *J. prakt. Chem.*, **102**, 222 (1867).

silver salt method to get what was apparently a crude amyl ether, described by him as a "heavy oil." Salkowski and Rehs[3] made the methyl ether by the same method, but obtained a relatively pure product. Willgerodt[4] used a different method, the reaction of the corresponding alcohol, under the influence of an alkali, with dinitrochlorobenzene, and obtained thereby the methyl and ethyl ethers as pure, crystalline compounds, but the propyl and isoamyl ethers as brown oils and apparently, in the light of the data of the present paper, only crude preparations. Thus the *n*- and isopropyl, *n*- and isobutyl, *n*- and isoamyl, *n*-hexyl, and *n*-heptyl ethers of 2,4-dinitrophenol, described in the following paragraphs, are new compounds.

Experimental

Alkyl Iodide–Silver Salt Reaction.—The silver dinitrophenol was made by two methods as follows: (1) 20 g. of sodium dinitrophenol (Eastman Kodak Co.) was dissolved in 150 cc. of water, and 16 g. of silver nitrate in 100 cc. of water was gradually added with stirring while both solutions were at the boiling temperature. The silver dinitrophenol was filtered from the chilled solution, recrystallized from water, air-dried in the dark, and pulverized. (2) 20 g. of dinitrophenol (Eastman Kodak Co.) was boiled with 500 cc. of water, and the hot, supernatant solution digested at 100° for one hour with an excess of freshly precipitated and washed silver carbonate. The solution was then filtered while hot, chilled, and the filtrate used again to dissolve more dinitrophenol. The crystals were air-dried, extracted with toluene to remove any dinitrophenol present, and again air-dried.

To a weighed amount of this dried and pulverized silver salt contained in a flask provided with a reflux condenser, was added, in small portions, somewhat more than an equimolecular weight of the respective alkyl iodide (Eastman). Cooling was required in some cases during the first additions, but, to complete the reaction, heating on a water-bath for a number of hours was necessary. At the end of this time the alcohol-soluble portion of the reaction mixture was extracted with successive portions of boiling 95% alcohol, leaving a residue of silver iodide. The alcohol and the excess of alkyl iodide were then distilled off, and the residue taken up with ether. The ether solution was washed free from any dinitrophenol present by means of dilute alkali, traces of alkali removed with dilute acid, and the acid washed out with water. After the ether and other easily volatile substances in this washed ether layer had been removed by vacuum distillation from a boiling water-bath, the residue containing the ether of dinitrophenol was fractionally distilled under 1–2 mm. pressure. The main fraction was twice crystallized from 95% alcohol, and refractionated at the high vacuum.

Alcohol–Dinitrochlorobenzene Reaction.[4]—This was used only for the preparation of the isopropyl ether. The procedure of Willgerodt[4] as well as the modifications of Holleman and Wilhelmy[5] and Vermeulen[6] were found to be quite inadequate for a product suitable for pharmacological use, principally because of the high content of the toxic dinitrochlorobenzene, and were, accordingly, modified greatly.

To 50 g. of dinitrochlorobenzene (Eastman) and 250 cc. of isopropyl alcohol (98–99%, Eastman) contained in a flask provided with a reflux condenser, was added 25 cc. of potassium hydroxide solution (80%) dropwise while the solution was kept at the boiling temperature and well stirred. After the alkali had been added, the heating and stirring were continued for one-half hour. At the end of this time, the solution was made acid and the excess of isopropyl alcohol was distilled off, leaving a residue of potassium chloride and the impure isopropyl ether. This last was extracted with ether and the free dinitrophenol present was washed out of the ether solution by means of dilute alkali. After an acid and water washing, the ether was evaporated and the residue fractionally distilled under 1–2 mm. pressure. The main fraction, with a distilling range of 2–3° was again dissolved in ether, and washed with alkali, acid and water as before. The residue, after evaporation of the solvent, was then crystallized from 95% alcohol, dried, pulverized, and extracted with petroleum ether until chlorine-free in order to remove the last traces of dinitrochlorobenzene. The yield of the purified isopropyl ether was 40% of the theoretical based on dinitrochlorobenzene.

Properties and Nitrogen Analyses of the Ethers.—All of these ethers were of a pale yellow color, heavier than and very difficultly soluble in hot and cold water, easily soluble in hot and somewhat difficultly soluble in cold 95% alcohol. The thermometer readings given below are corrected values.

***n*-Propyl,** b. p. 172–175° (2 mm.), m. p. 30.5–31.0°; long, prismatic needles from alcohol. Found: N, 12.63. Calcd.: N, 12.40.

Isopropyl, b. p. 152–156° (0.75 mm), m. p. 53.4–53.6°; long, flat needles and plates. Found: N, 12.39. Calcd.: N, 12.40.

***n*-Butyl,** b. p. 178–180° (2 mm.), m. p. 1.5–1.8°; irregular leaflets. Found: N, 11.21. Calcd.: N, 11.67.

Isobutyl, b. p. 152–154° (1 mm.), m. p. 30.3–31.5°; irregular leaflets. Found: N, 11.52. Calcd.: N, 11.67.

***n*-Amyl,** b. p. 186–188° (2 mm.), m. p. 0–1.0°; irregular leaflets with a tendency to prismatic form. Found: N, 10.62. Calcd.: N, 11.03.

Isoamyl, b. p. 175–178° (1 mm.), m. p. 9.5–10.0°; irregular leaflets. Found: N, 10.43. Calcd.: N, 11.03.

***n*-Hexyl,** b. p. 202–205° (2.5 mm.), m. p. 4.2–4.6°; irregular leaflets. Found: N, 10.26. Calcd.: N, 10.45.

***n*-Heptyl,** b. p. 192–194° (1 mm.), m. p. 16.4–16.5°; fine, sharp needles. Found: N, 9.93. Calcd.: N, 9.93.

Effect on the Metabolic Rate of Rats.—Two determinations of the basal rate were made following eighteen to twenty hours of fasting. The ether was then fed, and the metabolic rate

(3) H. Salkowski and G. Rehs, *Ber.*, **7**, 370 (1874).

(4) C. Willgerodt, *ibid.*, **12**, 762 (1879).

(5) A. F. Holleman and G. Wilhelmy, *Rec. trav. chim.*, **21**, 432 (1902).

(6) H. Vermeulen, *ibid.*, **25**, 12 (1906).

determined[7] at hourly periods for six hours. The first or preliminary hour necessary for equilibrium to be attained could not be utilized. Body surface areas used in calculating the metabolic rate per square meter were derived by the use of Lee's formula.[8] A trap containing 5% sulfuric acid was inserted in the closed circuit of the apparatus to remove ammonia from the circulating air. Determinations of the excreted ammonia were made in the case of a number of ethers.

The more gradual effect of the ethers as compared with dinitrophenol itself in stimulating the metabolic rate is shown in Table I. The moderate effect of the isopropyl ether, the relatively small amount of ammonia formed presumably from liver damage, and its ease of preparation, led to its selection for the prolonged feeding and toxicity tests described below.

TABLE I

AVERAGE STIMULATION OF METABOLIC RATE ABOVE BASAL RATE BY ALKYL ETHERS OF 2,4-DINITROPHENOL

	No. of rats used	Dose per kg., mg.	Increase in M. R., %, over basal rate at 1.5 hr.	2.5 hr.	3.5 hr.	4.5 hr.	5.5 hr.	NH_3 formed per kg. per 24 hr., mg.
Dinitrophenol	5	10	34	30	20	17	17	
Ether								
n-Propyl	4	80	0	6	9	13	15	144
Isopropyl	5	80	3	7	11	19	22	20
n-Butyl	4	80	4	9	13	18	26	
Isobutyl	2	80	2	10	15	19	12	
n-Amyl	4	80	0	4	14	19	28	148
Isoamyl	3	80	1	4	6	7	10	
n-Hexyl	3	80	2	1	0	1	2	
	7	160	9	11	7	7	8	363
n-Heptyl	4	160	7	10	21	26	33	416

Prolonged Feeding Experiments on Rats with the Isopropyl Ether of Dinitrophenol.—Twelve female rats (av. wt. 203 g.) were maintained on a diet of fox chow[9] to which 0.1% of the isopropyl ether had been added. This addition was made by stirring the ether solution of the isopropyl ether into the chow, and allowing the ether to evaporate. The average daily intake of isopropyl ether was found to be about 70 mg. per kg. of body weight. The basal metabolic rate of this group of rats at the end of one month was 10% more than that of the control group of 6 female rats (av. wt. 193 g.) that was fed the same diet without the addition of the isopropyl ether. The experiment was continued for eight months longer without discernible effect on the appearance of the animals. The rats were then sacrificed and necropsied.[10] Macroscopic examination showed a smaller amount of fat in general in the isopropyl group, but otherwise no difference could be discerned.

Effect of Massive Doses of the Isopropyl Ether on Rats.—Six rats were fed the isopropyl ether in doses of 1 g. per kg. of body weight. At the end of forty-eight hours the increase in their metabolic rate over the basal averaged 84% in 2 determinations per rat. On the third and fourth days after they were dosed these rats died in the characteristic dinitrophenol rigor. The loss in body weight at death was 15%. Necropsy showed a partial disappearance of fat, and marked pulmonary congestion, but otherwise no gross abnormalities.

For purposes of comparison, 4 rats were fed 80 mg. of dinitrophenol per kg. All died within an hour.

Intestinal Hydrolysis of the Isopropyl Ether.—The question arises as to whether the effect of the ethers on the metabolic rate is due to the pharmacological action of the ethers themselves or to free dinitrophenol formed by the hydrolysis of the ethers. This remains unanswered except for the fact that by the use of Guerbet's test[11] free dinitrophenol was found in the bile and intestinal (colon) contents of the dog two days after fatal, massive dosing with the isopropyl ether.

Summary

The following new alkyl ethers of 2,4-dinitrophenol were prepared: *n*- and isopropyl, *n*- and isobutyl, *n*- and isoamyl, *n*-hexyl and *n*-heptyl. The methods of preparation and a few of the properties of these ethers are described.

These ethers were found to exert a much more gradual effect in stimulating the metabolic rate of rats than dinitrophenol itself.

BALTIMORE, MARYLAND RECEIVED JULY 19, 1940

(7) Wesson, *J. Nutrition*, **3**, 503 (1931); *J. Biol. Chem.*, **102**, 303, footnote 2 (1933).

(8) Lee, *Am. J. Physiol.*, **89**, 24 (1929).

(9) Ralston-Purina Co., Minneapolis.

(10) Necropsies by Veader Leonard.

(11) Guerbet, *Ann. physiol. physicochim. biol.*, **8**, 100 (1932).