ice-water bath and alkaline sodium hypochlorite solution[25] (14.9%, 1550 ml.) added dropwise with stirring so that the mixture refluxed gently. After addition of the sodium hypochlorite solution (45 minutes) the mixture was stirred at 20° for 12 hr. The organic layer was then separated, the aqueous layer extracted with methylene chloride and the combined organic solutions washed with brine and dried over Drierite. Removal of the solvent gave a brown oil (170 g.) which was dissolved in light petroleum (b.p. 20–40°, 500 ml.) and kept at 0° overnight. Unchanged thiourea (23 g.) which separated was removed by filtration and the clear solution was concentrated to an oil which was distilled in a short path molecular still at 45–50° (5×10^{-4} mm.) to give the carbodiimide as a pale yellow oil (104.9 g., 67%) $n^{19}D$ 1.4861. An analytical sample was redistilled at 45° (5×10^{-4} mm.); $n^{22.5}D$ 1.4862. *Anal.* Calcd. for $C_{14}H_{27}N_3$: C, 70.80; H, 11.47; N, 17.71. Found: C, 70.64; H, 11.46; N, 17.63.

The methiodide (IV) was obtained by dissolving the carbodiimide (104 g.) in light petroleum (b.p. 20–40°, 500 ml.) and treating with methyl iodide (100 ml.) at 0° for 7 days. The methiodide separated as an oil which was redissolved in acetone and reprecipitated with light petroleum. This process was repeated until an aqueous solution of the heavy oil was neutral.

Reaction of AMP with 1-Cyclohexyl-3-(γ-diethylaminopropyl) Carbodiimide Methiodide (IV).—AMP (10 mg., 0.0275 m*M*) in a mixture of water (0.1 ml.) and pyridine (0.6 ml.) was treated with the diimide (379 mg., 1.0 m*M*) at 20°. The reaction in the homogeneous mixture was followed chromatographically in solvent A, nucleotide containing spots being eluted with dilute hydrochloric acid (0.1 *M*) and estimated spectrophotometrically. Reaction was complete in 1–2 hr., P^1P^2-diadenosine 5′-pyrophosphate (62%) being the only new product (R_f, 0.2, AMP, 0.3). Under otherwise identical conditions the reaction in water (0.35 ml.)-pyridine (0.35 ml.) and water (0.6 ml.)-pyridine (0.1 ml.) gave 25 and 18%, respectively, of the pyrophosphate.

The Reaction of AMP and Orthophosphoric Acid with IV.—AMP (36.5 mg. of the monohydrate, 0.1 m*M*) and 85% orthophosphoric acid (116 mg., 1.0 m*M*) in water (0.42 ml.)-pyridine (2.52 ml.) was treated with the diimide (3.79 g., 10.0 m*M*) at 20° (cooling required, initially) for 1 hr. Solvent was removed *in vacuo* at 20° and the residual gum taken up in acetone (100 ml.) containing barium iodide (2.136 g. of the dihydrate, 5.0 m*M*). After keeping the mixture at 0° overnight, the precipitate which separated was spun down, washed with acetone and dried. The dry white powder was suspended in water and stirred with I.R.-120 ion-exchange resin (sodium form). The resulting solution was made up to 10 ml. with water and an aliquot (2 ml.) chromatographed on an analytical ion-exchange column as described before. The recovery of optical density was 50%; AMP, 28; ADP, 15; and ATP, 56%.

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VANCOUVER 8, B. C., CANADA

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[CONTRIBUTION FROM THE NUTRITION AND PHYSIOLOGY DEPARTMENT, RESEARCH DIVISION, AMERICAN CYANAMID CO.]

Riboside Derivatives of 6-Methyl-*asym*-triazine-3,5(2,4)-dione[1]

BY ROSS H. HALL

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The chemical synthesis of 2-D-ribofuranosyl-6-methyl-*asym*-triazine-3,5(2,4)-dione is described. The synthesis of the corresponding 4-ribosyl and 2,4-diribosyl derivatives and their identification is also reported. A facile ring opening of the 4-substituted derivatives is described.

Various investigators have synthesized analogs of the naturally occurring pyrimidines for use as experimental anti-tumor compounds. The suggestion has been made recently that the nucleosides of such compounds may be more effective than the free bases.[2] A publication[3] supporting this concept demonstrated the value of the deoxyriboside of 6 - methyl - *asym* - triazine - 3,5(2,4) - dione (azathymidine) as an inhibitor of deoxyribonucleic acid (DNA) synthesis in contrast to the inertness of the free base. The deoxyriboside is obtainable at the present time only by enzymic means,[3] but in view of the evidence of Roll, *et al.*,[4] and Rose and Schweigert[5] that facile conversion of ribosides to deoxyribosides occurs within living cells it was decided to synthesize 6-methyl-*asym*-triazine-3,5-(2,4)-dione riboside *via* chemical means in order to evaluate its use as an anti-cancer agent. This paper describes the synthesis of a mixture of ribosides, their separation and identification.

A mercury salt of 6-methyl-*asym*-triazine-3,5-(2,4)-dione (VIII) was prepared and this was condensed with 1-chloro-2,3,5-tri-*O*-benzoyl-D-ribose,[6] according to the procedure of Davoll and Lowy.[7] The resulting mixture of products (65–70% yield) was separated on alumina and silicic acid columns to yield three products, IV, V and VI.[8] Each of these compounds was catalytically debenzoylated in anhydrous media by sodium methoxide to yield the three riboside derivatives I, II and III. These three derivatives also were obtained by first debenzoylating the condensation mixture, then separating the free nucleosides by partition chromatography on Celite. The analytical data indicated that two of the compounds were monoribosyl derivatives of 6-methyl-*asym*-triazine-3,5(2,4)-dione, while the third was a diribosyl derivative;

(1) This base is often referred to as 6-azathymine.

(2) Examples of the effectiveness of nucleosides over the corresponding free bases as antimetabolites are: 5-bromodeoxyuridine *versus* 5-bromouracil, T. J. Bardos, G. M. Levine, R. R. Herr and H. L. Gordon, THIS JOURNAL, **77**, 4279 (1955); 6-azauridine *versus* 6-azauracil, R. E. Handschumacher, *Federation Proc.*, **16**, 191 (1957); A. D. Welch and R. Schindler, *Science*, **125**, 548 (1957); azathymidine *versus* azathymine, W. H. Prusoff and A. D. Welch, *J. Biol. Chem.*, **218**, 929 (1956), and W. H. Prusoff, L. G. Lajtha and A. D. Welch, *Biochim. et Biophys. Acta*, **20**, 209 (1956); W. H. Prusoff, *J. Biol. Chem.*, **226**, 901 (1957).

(3) Ross H. Hall and R. Haselkorn, THIS JOURNAL, **80**, 1138 (1958).

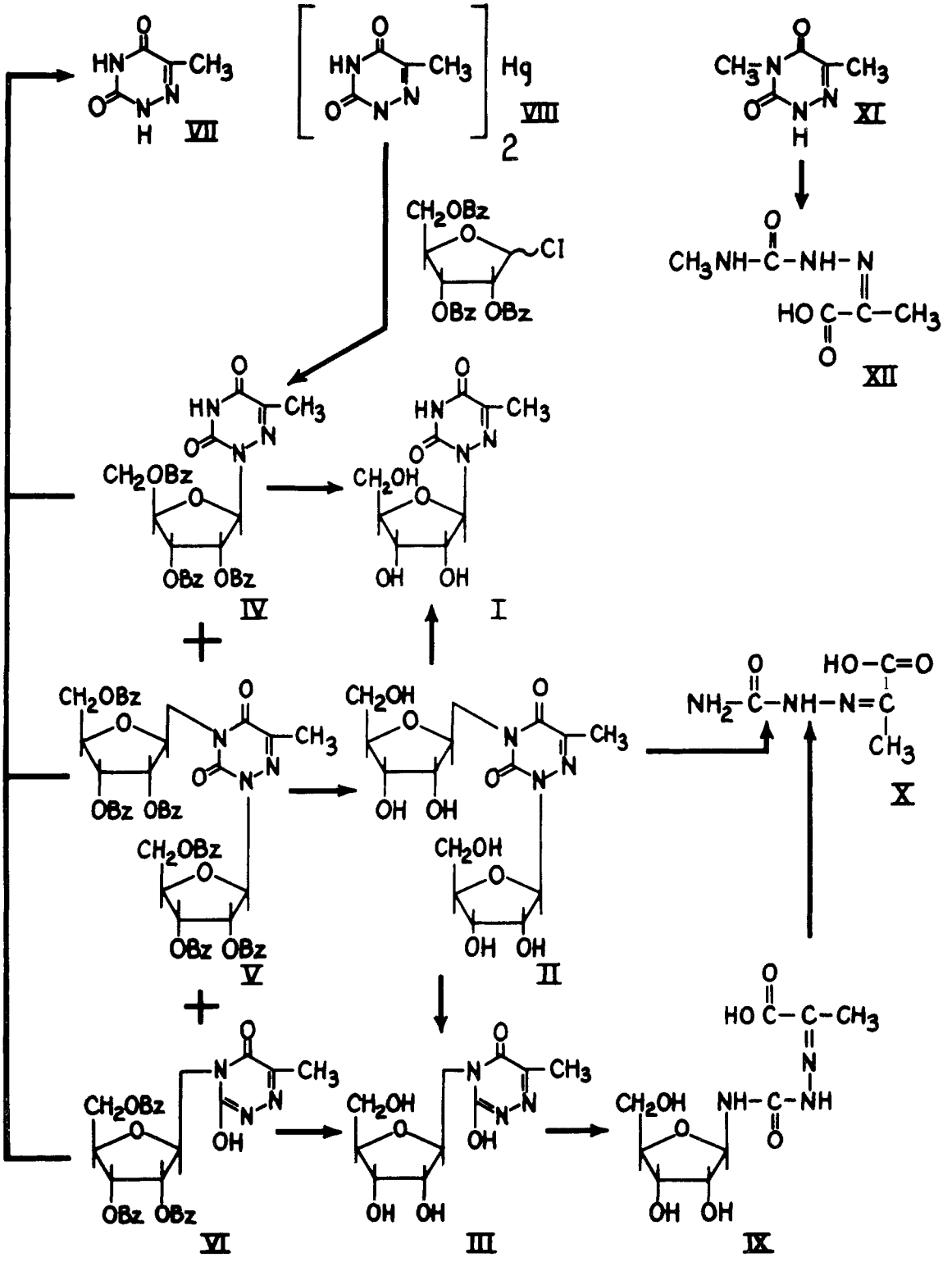
(4) P. Roll, H. Weinfeld, E. Carroll and G. B. Brown, *J. Biol. Chem.*, **220**, 439 (1956).

(5) I. A. Rose and B. S. Schweigert, *ibid.*, **202**, 635 (1953).

(6) R. K. Ness, H. W. Diehl and H. G. Fletcher, THIS JOURNAL, **76**, 763 (1954).

(7) J. Davoll and B. A. Lowy, *ibid.*, **74**, 1563 (1952).

(8) The glycosidic linkages of all formulas are written as if they were in the β-configuration, although there is no evidence to support this.



evidence for location of the sugar residues on the base will be given.

Structure III, 4-D-ribofuranosyl-6-methyl-*asym*-triazine-3,5(2,4)-dione, was assigned after studying the properties of a model compound, 4,6-dimethyl-*asym*-triazine-3,5(2,4)-dione (XI). This compound was prepared by methylation of VIII with methyl iodide. Attachment of the methyl group to N-4 was demonstrated by heating the compound with strong alkali. This caused scission of the ring giving the 4-methylsemicarbazone of pyruvic acid, XII, which was identified by comparison with an unambiguously synthesized sample. The ultraviolet absorption spectra of III closely resembled that of the model compound XI (Table I). In particular the alkaline batho-

chromic shift was characteristic. Shugar and Fox[9] reported a similar alkaline bathochromic shift for 3-methyluracil but not for 1-methyluracil. The ultraviolet absorption peak of the other monoriboside, compound I, underwent a shift to shorter wave lengths in alkali; therefore, of the two monoribosides only compound III merited consideration as a four substituted *asym*-triazine.

TABLE I

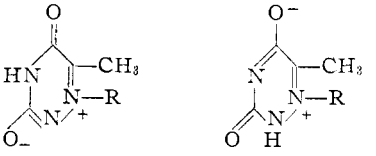
ULTRAVIOLET ABSORPTION OF COMPOUNDS

Derivative of 6-methyl-*asym*-triazine-3,5(2,4)-dione	0.1 *N* HCl λ_{max}	0.1 *N* HCl ϵ_{max} (× 1000)	—H₂O— λ_{max}	—H₂O— ϵ_{max} (× 1000)	0.1 *N* NaOH λ_{max}	0.1 *N* NaOH ϵ_{max} (× 1000)
2-Ribosyl-	262	4.95	262	5.10	251	5.4
2-Deoxyribosyl-	262	5.80	262	5.80	251	6.2
2,4-Diribosyl-	267	4.3	267	4.15	..	..
4-Ribosyl-	264	5.1	264	5.15	304	..
4-Methyl-	260	5.2	260	5.0	298	7.9
4-Ribosylsemicarbazone of pyruvic acid	257	4.6	248	6.7	248	6.0
Semicarbazone of pyruvic acid	257	7.6	248	7.55	248[a]	7.55
Semicarbazone of pyruvic acid from 2,4-diribosyl derivative	257		248		248	
Semicarbazone of pyruvic acid from 4-ribosyl-derivative	257	7.6	248	7.5	248	7.5
2,4-Dimethyl-	274	6.9	273	6.4	273	6.3

[a] In 1 *N* alkali λ_{max} is 279, ϵ_{max} is 7,600. This denotes a new dissociation with *pk* greater than 13.

Further evidence as to the location of the ribose in compound III was forthcoming on studying the infrared spectra of compounds III and XI. Both these compounds had the same general absorption pattern. Of particular significance was the pattern in the carbonyl region; here the pattern of the two derivatives was identical. There were two bands at 5.78 and 6.00 μ, respectively. This indicated that compound III, like XI, was in the diketo form. Thus compound III was not an O-glycoside, a conclusion supported by the fact that this compound was relatively stable in boiling 1.0 *N* hydrochloric acid.[10]

One other possibility for compound III that was evaluated was attachment of the sugar at position one. This would mean an internally compensated quaternary compound, in order that the analytical data should apply. Two possible structures are



R = 2-deoxy-D-ribose

A positively charged linkage such as this would be expected to decompose easily in acid or alkali analogous to nicotinamide riboside.[11] Compound III, although relatively acid-stable, did undergo a rapid ring scission between atoms four and five in weak alkali, demonstrating a fundamental instability in this medium. This reaction is described in detail below. Such an occurrence is similar to that reported for the breakdown of 3,5′-cyclo-6-dimethylamino-9-(3′-deoxy-β-D-ribofuranosyl)-purine-2′,3′-carbonate methanesulfonate which has a charged nitrogen atom in the pyrimidine ring.[12] However, the infrared spectrum of compound III does indicate the presence of two keto groups, so that a charged structure like those above does not seem reasonable for this compound.

The instability of compound III in alkali was studied; at a *p*H value of 10, in a few minutes, it added one molecule of water undergoing ring opening to a ribofuranosyl semicarbazone of pyruvic acid, IX. Compound IX had an ultraviolet absorption spectra identical with that of the semicarbazone of pyruvic acid (Table I). Thus the sugar residue was in such a position as not to modify the spectral characteristics; that is to say, it could be either at position two or four of the semicarbazone but in accord with structure III would have to be at position four. It should be pointed out that if compound III contained the sugar residue at N-1 as discussed above, a rapid alkaline rearrangement of the sugar from atoms one to two would have had to be realized. There is little evidence that such rearrangements occur. That compound IX was indeed a semicarbazone of pyruvic acid, X, was confirmed by removal of the sugar residue by mild acid hydrolysis. The hydrolytic product was identified by comparison with an authentic sample.

The alkaline lability of the bond between atoms four and five of compound III has its analogy in the chemistry of dihydrothymine and dihydrouracil.[13] Mild alkaline treatment produces the corresponding ureido compound in each case. As noted above, the 4-methyl substituted *asym*-triazine required vigorous alkaline treatment in contrast to the labile 4-glycosyltriazine for ring scission, but in each case scission did occur in the same location. In summation, all the spectral and hydrolytic data are in accord with formulation of compound III as 4-D-ribofuranosyl-6-methyl-*asym*-triazine-3,5(2,4)-dione.

The structure of compound I was arrived at by a process of elimination. Establishment of the position of the sugar residue in compound III eliminated N-4 as the point of attachment for I. Much of the same argument for not considering III as an O-ribosyl derivative or an N-1 substituted derivative also applies to compound I. Mainly, this compound was relatively stable in boiling 1.0 *N* alkali and acid. Therefore, compound I was considered to be 2-D-ribofuranosyl-6-methyl-*asym*-triazine-3,5(2,4)-dione. The enzymatically prepared deoxyriboside[3] had an ultraviolet absorption pattern identical with that of I (Table I) and was therefore presumed to be 2-(2′-deoxy-D-ribofuranosyl)-6-methyl-*asym*-triazine-3,5(2,4)-dione.

(9) D. Shugar and J. J. Fox, *Biochim. et Biophys. Acta*, **9**, 199 (1952).

(10) P. A. Levine and H. Sobotka studied the lability of pyrimidine O-glycosides in acid and base; *J. Biol. Chem.*, **65**, 469 (1925).

(11) N. A. Hughes, G. W Kenner and Sir Alexander Todd, *J. Chem. Soc.*, 3733 (1957); T. P. Singer and E. B. Kearney, *Adv. in Enzymol.*, **15**, 79 (1954).

(12) B. R. Baker and J. P. Joseph, THIS JOURNAL, **77**, 15 (1955).

(13) R. M. Fink, C. McGaughey, R. E. Cline and K. Fink, *J. Biol. Chem.*, **218**, 1 (1956); R. D. Batt, J. K. Martin, J. Ploesser and J. Murray, THIS JOURNAL, **76**, 3663 (1954).

From microanalytical data, compound II appeared to be a diriboside of VII. The location of the riboside residues at position two and four were determined as follows: Mild acid hydrolysis resulted in a quantitative yield of compound I. Vigorous acid hydrolysis seemed to remove the sugar residue from position one slightly faster than from position three because just prior to complete hydrolysis to the free base VII it was possible to demonstrate a small quantity of compound III on paper strips. The identification of this hydrolytic product was confirmed by R_f value on paper strips, its characteristic ultraviolet absorption spectra, and its distinctive behavior to alkali. Like compound III, compound II was extremely labile to alkali and underwent ring opening to give a product which after acid hydrolysis appeared to be identical with the semicarbazone of pyruvic acid, X.

The main purpose of this problem was the synthesis of 2-D-ribofuranosyl-6-methyl-*asym*-triazine-3,5(2,4)-dione (I) and when the chemistry of the three ribosyl derivatives had been established, a more direct pathway to this end became evident. After the initial condensation, the mixed benzoylated isomers were debenzoylated; care was taken not to permit contact with aqueous alkali. The mixture of nucleosides was treated first with dilute acid to hydrolyze II to I then with alkali to convert III to IX. The problem then became one of separation of I from IX, easily accomplished by ion exchange chromatography. In this manner compound I was obtained in 41% over-all yield. The compound was a strong complexing agent so that it was necessary to remove bound metal ions by sulfide precipitation prior to final isolation of the product.

Experimental[14]

Mercury Salt of 6-Methyl-*asym*-triazine-3,5(2,4)-dione (VIII).—6-Methyl-*asym*-triazine-3,5(2,4)-dione,[15] (25.4 g., 0.2 mole) was dissolved in 200 cc. of 1 *N* alkali. Mercuric chloride (27 g., 0.1 mole) dissolved in 200 cc. of water was added slowly to the above solution. The mixture was chilled overnight; the crystals were collected and washed with water until free of chloride ion; yield 40 g. (84% of theory).

Anal. Calcd. for $C_8H_8N_8O_4Hg \cdot H_2O$: C, 20.87; H, 2.14; N, 17.85. Found: C, 20.49; H, 1.94; N, 17.92.

Condensation of the Mercury Salt VIII with 2,3,5-Tri-*O*-benzoyl-1-chloro-D-ribofuranose.—A solution of 19.2 g. (38 mmoles) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose[6] in 400 ml. of absolute ether was saturated with hydrogen chloride at 0°. After 4 days the ether was evaporated and the residue dissolved in 100 cc. of toluene. The toluene was distilled *in vacuo* and the residue redissolved in toluene to be re-evaporated. This process was repeated twice more whereupon the residue was dissolved in 50 cc. of toluene and added to a previously set up reaction as follows: Compound VIII (9.4 g., 20 mmoles) was suspended in 300 cc. of anhydrous toluene in a 3-neck flask equipped with a stirrer and condenser. About 100 cc. of toluene was distilled off before addition of the above sugar solution. The reaction mixture was stirred and refluxed under a positive pressure of dry nitrogen for 12 hours.

The hot solution was filtered and evaporated *in vacuo*. The residue was dissolved in 300 cc. of chloroform and the solution extracted twice with 30% potassium iodide solution, twice with 1 *M* sodium Versenate solution, once with 0.1 *N* hydrochloric acid and finally with water. Evaporation of the chloroform solution left 21.2 g. of a fluffy solid. This consisted of a mixture of the three isomeric nucleosides and unreacted ribose, all as the tribenzoylated derivatives. It is termed mixture A.

Separation of Mixture A on Activated Alumina.—Merck reagent grade alumina (acid-washed) was used directly from the bottle in preparing a column 7.1 cm.^{2} × 41 cm. (300 g.), with benzene as the initial solvent. Mixture A (10.6 g.) was dissolved in 40 cc. of benzene and run on the column. The column was washed with the following solvents at a flow rate of 10 cc./minute: 2.2 l. of benzene, which eluted 1.5 g. of unreacted sugar product (m.p. 69–72°); 2 l. of 5% ethyl acetate in benzene which eluted 3.37 g. of V and a ribose impurity; 3 l. of 10% ethyl acetate in benzene which eluted 2.8 g. of IV; 2.2 l. of 25% ethyl acetate in benzene which eluted 3.1 g. of VI and IV.

Purification of VI was achieved by running a second column on alumina of greater activity. The above alumina (130 g.) was heated at 180° for 24 hours and used to prepare a column 3.2 cm.^{2} × 50 cm. A gradient elution between 1 l. of benzene and 1 l. of ethyl acetate was set up with increasing amounts of ethyl acetate. Compound VI (2 g.) was eluted before IV. Compound VI was crystallized from a mixture of methanol and petroleum ether to give crystals, m.p. 155.5–156°.

Anal. Calcd. for $C_{30}H_{25}N_3O_9$: C, 63.02; H, 4.41; N, 7.35. Found: C, 62.84; H, 4.57; N, 7.48.

Both compounds IV and V were contaminated with ribose impurities. They were purified on silicic acid columns as follows. Mallinckrodt, 100 mesh, chromatographic silicic acid was heated at 200° for 16 hours. Individual columns were prepared by suspending 20 g. of silicic acid in 50 cc. of chloroform and pouring the slurry into a 20-mm. diameter tube. Samples of IV and V weighing 0.30 g. were dissolved in 2 cc. of chloroform and run onto their respective column. Progress down the columns of the derivatives was easily followed by the opaqueness of the bands. Compound V (200 mg.) was eluted with 5% methanol in chloroform and compound IV (187 mg.) was eluted with 100% chloroform from its column. The samples were isolated as glasses.

Anal. Calcd. for $C_{30}H_{25}N_3O_9 \cdot 3H_2O$: C, 57.58; H, 5.00; N, 6.72. Found IV: C, 57.82; H, 5.00; N, 6.25. Calcd. for $C_{56}H_{45}N_3O_{16}$: C, 66.21; H, 4.46; N, 4.13. Found V: C, 66.75; H, 4.71; N, 3.81.

Catalytic Debenzoylation.—The following procedure is applicable to either a mixture of the benzoylated isomers or to the pure compounds. Mixture A (10.2 g.) was dissolved in 70 cc. of absolute methanol and refluxed for 4.25 hours during which time 5 cc. of 1 *N* sodium methoxide in methanol was added in 1-cc. portions at 0.75-hour intervals. The amount of sodium methoxide varied from run to run, but it was essential to maintain a *p*H of approximately 9–10. When the *p*H remained at this value for 0.5 to 0.75 hour without further addition of alkali, the reaction was considered finished. A spot check of the progress of the debenzoylation could be obtained by chromatography on paper (5% ammonia in water saturated 1-butanol). The free nucleosides have low R_f values (*ca.* 0.20) compared to the partially debenzoylated derivatives.

The reaction mixture was worked up by adding the calculated amount of semi-dry Dowex-50 (H^+) resin necessary to neutralize the added sodium methoxide. The mixture was filtered and the resin washed with methanol then with water. The combined washings and filtrate were diluted with more water and evaporated to near dryness. The residue was dissolved in 100 cc. of water and extracted thrice with ether in order to remove any benzoic acid. The aqueous layer was saturated with hydrogen sulfide and then charcoal added. After warming, the solution was filtered and the filtrate lyophilized to give 3.08 g. of a mixture of compounds I, II and III (very hygroscopic). This corresponds to a 65% yield of theory based on the amount of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose. This mixture is termed mixture B.

Partition Chromatography of Mixture B.—The solvent used in the following procedure was prepared by vigorously shaking together 500 parts of ethyl acetate, 100 parts of methanol and 200 parts of water (volumes). Two hundred grams of Celite 545 (washed with acid, then water and dried) was thoroughly mixed with 100 cc. of the lower phase and carefully tamped into a column 50 mm. in diameter using a

(14) All melting points are uncorrected.

(15) E. A. Falco, E. Pappas and G. H. Hitchings, THIS JOURNAL, **78**, 1938 (1956).

tightly fitting cork plunger. The column was 43 cm. high. Mixture B (2.1 g.) was dissolved in 10 cc. of the lower phase and mixed with 20 g. of Celite, which was then tamped on to the top of the column. The column was eluted with the upper phase at a flow rate of 225 cc./hr. The hold back volume (HBV) was 410 cc. The results were

HBV	Derivative eluted	Wt., g.
0–1.5	The 4-ribosyl, III	0.61
1.5–9.5	The 2-ribosyl, I	.43
12–14.5	The 2,4-diribosyl, II	.345

Of the three isomers, only compound III was obtained as a crystalline non-hygroscopic solid (0.4 g. crystallized from 2 cc. of hot 1:1 ethyl acetate–isopropyl alcohol yielded 0.354 g., m.p. 164–165°). The other two isomers were so hygroscopic as to prevent easy handling and were left as glasses.

Anal. Calcd. for $C_9H_{13}N_3O_6$: C, 41.70; H, 5.06; N, 16.21. Found III: C, 41.85; H, 5.22; N, 16.49. Calcd. for $C_9H_{13}N_3O_6 \cdot 3H_2O$: C, 34.49; H, 6.12; N, 13.42. Found I: C, 34.00; H, 6.26; N, 13.82. Calcd. for $C_{14}H_{21}N_3O_{10}$: C, 42.97; H, 5.41; N, 10.74. Found II: C, 43.40; H, 5.70; N, 10.13.

4-D-Ribofuranosylsemicarbazone of Pyruvic Acid, IX.—4-D-Ribofuranosyl-6-methyl-*asym*-triazine-3,5(2,4)-dione (III) (0.5 g., 1.93 mmoles) was dissolved in 25 cc. of 4 *N* ammonium hydroxide. After three hours, the solution was evaporated to dryness *in vacuo* and benzene added, then evaporated. This cycle was repeated twice to yield a fluffy hygroscopic glass; wt. 0.48 g. (89.5% of theory).

Anal. Calcd. for $C_9H_{15}N_3O_7$: C, 38.99; H, 5.45; N, 15.16. Found: C, 38.85; H, 6.00; N, 15.43.

Semicarbazone of Pyruvic Acid, X.—4-D-Ribofuranosylsemicarbazone of pyruvic acid (250 mg.) was dissolved in 25 cc. of 10% acetic acid and the solution was refluxed 6 hours. The solution was evaporated *in vacuo* to near dryness, water added and re-evaporated to a volume of 2 cc. On standing overnight there appeared 200 mg. of a crystalline material, m.p. 215–217°. The melting point of this compound was not depressed upon admixture with an authentic sample of semicarbazone of pyruvic acid.[16] Further, the R_f value of the two samples on paper strips were identical in several solvent systems (*n*-BuOH–H_2O–HOAc, 5:3:2; isopropyl alcohol–2 *N* HCl, 2:1; isopropyl alcohol–1% $(NH_4)_2SO_4$, 2:1; ETOAc satd. with 3% HOAc). Also the ultraviolet absorption pattern of the two samples was identical (Table I).

Anal. Calcd. for $C_4H_7N_3O_3$: C, 33.10; H, 4.86; N, 28.96. Found: C, 32.70; H, 5.04; N, 29.35.

Hydrolytic Behavior of 2,4-Di-D-ribofuranosyl-6-methyl-*asym*-triazine-3,5(2,4)-dione (II).—All R_f values in the following description were determined in the ethyl acetate–3% acetic acid system unless otherwise stated.

A. 1 N Hydrochloric Acid.—Forty mg. of compound II was dissolved in 3.5 cc. of 1 *N* hydrochloric acid and refluxed 22 hours. The solution was streaked on Whatman 3MM paper and developed in the above system. Three streaks were then visible under ultraviolet irradiation. The fastest moving component (70% of total) corresponded to VII, while the other two streaks (15% of total each) corresponded to compounds I and III, respectively.[17] Each streak was eluted and its ultraviolet adsorption determined at *p*H values of 1, 7 and 13. The λ_{max} values for each streak were identical with the λ_{max} values listed in Table I for the aforementioned compounds. A third criterion of identity for the hydrolytic product corresponding to compound III was the fact that when the eluted spot was made slightly alkaline and re-run on paper, a new compound corresponding to the 4-ribosylsemicarbazone of pyruvic acid was observed. Hence, that compound III is a hydrolytic product of compound II is fairly certain.

B. Acetic Acid.—Thirty mg. of compound II dissolved in 5 cc. of 20% acetic acid was refluxed 6 hours. Chromatographic analysis on paper showed that 85% of compound II had been converted to a new compound corresponding to the 2-ribosyl derivative I. This spot was eluted and its ultraviolet spectrum found to correspond exactly with that of compound I as listed in Table I.

C. Alkali Prior to Acid Hydrolysis.—Fifty mg. of compound II was dissolved in 5 cc. of dilute alkali (*p*H 10), then within a few minutes made 10% with respect to acetic acid. After refluxing 6 hours, examination of paper strips showed no trace of starting material and instead there were two new ultraviolet absorbing spots. The ultraviolet absorption spectra at *p*H values 1, 7 and 13, of each of the eluted spots was identical with that of the semicarbazone of pyruvic acid listed in Table I. The R_f value of the faster component (60% of total) corresponded to that of pyruvic acid semicarbazone in this solvent system as well as others (*n*-BuOH–HOAc, 5:3:2; isopropyl alcohol–2 *N* HCl, 2:1; isopropyl alcohol–1% $(NH_4)_2SO_4$, 2:1). The slower moving spot may have been the 2-ribosylsemicarbazone of pyruvic acid.

2-D-Ribofuranosyl-6-methyl-*asym*-triazine-3,5(2,4)-dione (I).—Mixture B (8.4 g.) was dissolved in 200 cc. of 20% acetic acid and refluxed 6 hours after which the solvent was removed *in vacuo*. The residue was dissolved in water which was evaporated. This was repeated thrice to ensure removal of acetic acid. The residue was dissolved in 50 cc. of water and treated with ammonia until a *p*H of 10.2 was reached. After 4 hours at room temperature the solution was run on to a column of Dowex-1 × 4 resin (200–400 mesh, formate), size 20 cm.[2] × 50 cm., previously equilibrated by passing through 5 l. of 0.01 *M* ammonium formate solution (*p*H 10.5). The column was washed first with 3 l. of 0.02 *M* ammonium formate buffer (*p*H 7.5) then with 24 l. of 0.02 *M* ammonium formate buffer (*p*H 5). The progress of elution was followed by reading the optical density at 260 mμ. The latter buffer removed the desired nucleoside while the 4-D-ribofuranosylsemicarbazone of pyruvic acid (IX) remained on the column.

The fractions containing compound I were combined and concentrated to 500 cc. *in vacuo*. The solution was filtered, then lyophilized. In the final stages of lyophilization the flask was warmed to 40° to drive off ammonium formate. Final traces of the salt were removed by dissolving the residue in a small quantity of water and relyophilizing. Compound I was obtained as a very hygroscopic glass; wt. 5.3 g. This corresponds to a 41% over-all yield based on the original amount of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose.

4,6-Dimethyl-*asym*-triazine-3,5(2,4)-dione (XI).—Ten grams (0.021 mole) of the mercury salt VIII was suspended in 177 cc. of toluene. After 35 cc. of toluene was distilled off 24 cc. (0.039 mole) of methyl iodide was added over a 5-hour period while stirring and refluxing the solution. The solution was refluxed a further 3 hours then cooled, filtered, and evaporated to dryness. The residue was dissolved in 100 cc. of chloroform and washed twice with 40 cc. of 30% potassium iodide solution and once with water. The residue, which was soluble in ethanol, methanol, chloroform, benzene and acetonitrile and insoluble in hexane, readily was purified by crystallization then sublimation, m.p. 157–158°, wt. 0.85 g. (15%).

Anal. Calcd. for $C_5H_7N_3O_2$: C, 42.55; H, 5.00; N, 29.78. Found: C, 42.87; H, 5.10; N, 30.25; neg. test for O–CH_3 (0.13%) (Zeisel); pos. test for N–CH_3 (7.88%) (Friedrich).

2,4,6-Trimethyl-*asym*-triazine-3,5(2,4)-dione.—Ten grams (7.8 mmoles) of 6-methyl-*asym*-triazine-3,5(2,4)-dione and 10 g. (17.5 mmoles) of potassium hydroxide were dissolved in 100 cc. of water and stirred at 60° while 19 cc. (20 mmoles) of freshly distilled dimethyl sulfate was added over a period of one hour. The *p*H was maintained in the alkaline range during the dimethyl sulfate addition by gradual addition of 30 mmoles of potassium hydroxide. After all the reactants were mixed, the mixture was stirred for two more hours at 60°, followed by reduction in volume to 85 cc. After chilling overnight 5.7 g. of crystals (47% of theory) were filtered off, m.p. 98–102°. This compound exhibited a double melting point. The material was crystallized from 20 cc. of water to give 4.8 g. of crystals melting at 82–85°. If the solidified melt in the melting point tube was remelted the melting point was now 101.5–103°.

Anal. Calcd. for $C_6H_9N_3O_2$: C, 46.45; H, 5.31; N, 27.10. Found: C, 46.65; H, 5.89; N, 27.14.

(16) G. Klein and W. Fuchs, *Biochem. Z.*, **213**, 40 (1929).

(17) When either compounds I or III were subjected to boiling 1 *N* hydrochloric acid for 16 hours, they were both approximately 90% hydrolyzed to VII as determined by paper chromatography. Boiling 2.5 *N* sodium hydroxide for two hours had no effect on I.

4-Methylsemicarbazone of Pyruvic Acid. A. From 4,6-Dimethyl-*asym*-triazine-3,5(2,4)-dione (XI).—One-half gram (3.55 mmoles) of XI was dissolved in 3.75 cc. of 1 *N* sodium hydroxide and 1.7 cc. of 6 *N* sodium hydroxide. The solution was heated on a steam-bath for 5 hours in a sealed tube after which it was diluted to 25 cc. with water. Paper chromatography in water-saturated 1-butanol showed about 20% of the starting material (R_f 0.75) and a new spot (R_f 0.15) containing the other 80% of ultraviolet-absorbing material. The semicarbazone of pyruvic acid has an R_f value of 0.02 in this system. The solution was passed through a column containing 15 cc. of Dowex-50 resin (H^+) which was then washed with 120 cc. of water. The effluents were concentrated to dryness and the residue (0.45 g.) crystallized from 25 cc. of boiling ethanol to yield 0.075 g., m.p. 228°. This precipitate was recrystallized from 2 cc. of boiling ethanol to yield 40 mg., m.p. 229–230°. The melting point of this sample was not depressed upon admixture with an authentic sample of 4-methylsemicarbazone of pyruvic acid prepared as described below.

Anal. Calcd. for $C_5H_9N_3O_3$: C, 37.73; H, 5.70; N, 26.41. Found: C, 38.20; H, 5.81; N, 26.55.

B. From Pyruvic Acid and 4-Methylsemicarbazide.—A mixture of 0.3 g. (3.4 mmoles) of pyruvic acid and 0.3 g. (3.4 mmoles) of 4-methylsemicarbazide[18] in 5 cc. of water was refluxed 1.5 hours. On cooling, 0.3 g. (56% yield) of a product melting at 234° was obtained. This precipitate was recrystallized from 34 cc. of hot ethanol to give a product of melting point 229–230°.

Anal. Calcd. for $C_5H_9N_3O_3$: C, 37.73; H, 5.70; N, 26.41. Found: C, 38.00; H, 5.92; N, 26.50.

(18) C. Vogelsang, *Rec. trav. chim.*, **62**, 5 (1943).

Paper Chromatography.—The solvent system was ethyl acetate saturated with 3% acetic acid, run in a descending fashion with ethyl acetate saturated water in bottom of tank. The spots were visualized under a Gates ultraviolet lamp (germicidal bulb).

Derivative of *asym*-triazine-3,5(2,4)-dione	*R*
4,6-Dimethyl, XI	0.93
2,4,6-Trimethyl	.90
6-Methyl, VII	.84
2-(2′-Deoxy-D-ribosyl)-6-methyl	.63
4-D-Ribosyl-6-methyl, III	.63
2-D-Ribosyl-6-methyl, I	.49
Semicarbazone of pyruvic acid, X	.29
2,4-Di-D-ribosyl-6-methyl, II	.20
4-Ribosylsemicarbazone of pyruvic acid, IX	.05

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[CONTRIBUTION FROM THE CHEMICAL CORPS, CHEMICAL RESEARCH DIVISION, CHEMICAL WARFARE LABORATORIES]

Organic Phosphorus Compounds. II.[1] Isomeric Alkyl Phosphoro- and Phosphonothioates

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O,O-Diethyl O-(2-ethylthioethyl) phosphorothioate (I)(Systox[3]) was prepared by the transesterification of triethyl phosphite with 2-ethylthioethanol and subsequent addition of sulfur to the resulting diethyl 2-ethylthioethyl phosphite. The O-(2-isopropylthioethyl) homolog of I was obtained in an analogous manner using 2-isopropylthioethanol. The transesterification with 2-alkylthioethanols was extended successfully to dialkyl methylphosphonites; the resulting O-alkyl O-(2-alkylthioethyl) methylphosphonites upon addition of sulfur yielded analogs of I containing a carbon-to-phosphorus bond. In several instances, the O-alkyl O-(2-alkylthioethyl) methylphosphonothioates were found to undergo thermal rearrangement to the corresponding S-(2-alkylthioethyl) isomers under conditions reported[4] for the analogous isomerization of I.

Introduction

The highly effective systemic insecticide O,O-diethyl O-(2-ethylthioethyl) phosphorothioate[3] (I) and some of its homologs were prepared by several investigators by the reaction of the appropriate alkylthioethanol or its sodium derivative with a suitable dialkyl phosphorothiochloridate, $(RO)_2P(S)Cl$.[4–7] The thiolo isomers of I and of its homologs of the type $(RO)_2P(O)SCH_2CH_2SR'$ (II) were obtained either by thermal isomerization[4] of the corresponding thiono isomers $(RO)_2P(S)OCH_2CH_2SR'$ (III) or by the reaction of a suitable trialkyl phosphite or dialkyl sodium phosphonate with a 2-alkylthioethyl thiocyanate.[6,8]

The inhibitory effect on cholinesterase enzymes increases by several orders of magnitude upon isomerization of O,O-dimethyl O-(*p*-nitrophenyl) phosphorothioate and its dialkyl homologs to the corresponding O,O-dialkyl S-(*p*-nitrophenyl) phosphorothioates[9] and the systemic insecticidal activity of the commercial Systox[3] seems to be dependent upon its contamination by the more active isomer $(C_2H_5O)_2P(O)SCH_2CH_2SC_2H_5$ (IV).[4] Since samples of the thiono compounds III, prepared by any of the previously reported methods[5–7] and purified by ordinary high vacuum distillation were always found to contain considerable amounts of the isomeric thiolo derivative, the preparation of the III, free from contamination with the isomeric II, became desirable. The previously reported

(1) Paper I of this series: F. W. Hoffmann, T. C. Simmons and L. J. Glunz, III, THIS JOURNAL, **79**, 3570 (1957).

(2) To whom inquiries about this paper should be addressed.

(3) Compound I is marketed by Chemagro Corporation, New York, N. Y., under the trade-name Systox.

(4) T. R. Fukuto and R. L. Metcalf, THIS JOURNAL, **76**, 5103 (1954).

(5) G. Schrader, U. S. Patent 2,571,989 (1951).

(6) Farbenfabriken Bayer, German Patent 850,677 (1952).

(7) Ya. A. Mandelbaum, N. N. Mel'nikov and V. I. Lomakina, *Zhur. Obshchei Khim.*, **26**, 2581 (1956); *C.A.*, **51**, 1825e (1957).

(8) G. Schrader, U. S. Patents 2,597,534 (1952) and 2,640,847 (1953); German Patents 926,488 and 947,367 (1956).

(9) R. L. Metcalf and R. B. March, *J. Econ. Entomol.*, **46**, 288 (1953).