ms, 3.42 m, 6.36 vvs, 7.04 s, 7.26 vs, 7.40 vs, 7.81 vs, 8.07 vs, 8.35 s, 8.72 s sh, 9.16 s, 9.38 s, 9.67 vs, 10.37 m, 10.97 ms, 11.30 s, 12.07 ms, 13.04 ms, 14.47 s. The peak at 6.36 $\mu$ corresponded to the asymmetric $NO_2$ stretching vibration in compounds containing the $-CH_2NO_2$ group.

On treatment of the nitro ether with a sodium hydroxide solution, much fluoride ion was liberated, a behavior similar to that for ethers of the type $ROCF_2CH_2Cl$.[12]

**The Reaction of 1,1-Difluoro-1-iodo-2-nitroethane with Methanol.**—$ICF_2CH_2NO_2$ (10.3 g.) was refluxed with 50 ml. of methanol for 4 hr. after which the excess methanol, as well as some methyl iodide and hydrogen iodide formed in the reaction, were boiled off at atmospheric pressure. The residue was dissolved in methylene chloride, washed with aqueous sodium bisulfite to remove iodine, dried over anhydrous magnesium sulfate, and distilled. The pure ether, 1,1-difluoro-1-methoxy-2-nitroethane, b.p. 57° (22 mm.), was obtained in 95% yield.

*Anal.* Calcd. for $C_3F_2H_5NO_3$: C, 25.54; H, 3.57; F, 26.93; N, 9.93. Found: C, 25.51; H, 3.41; F, 26.44; N, 9.84.

The infrared spectrum of $CH_3OCF_2CH_2NO_2$ (liquid) displayed the following absorption bands (in microns): 3.22 m, 3.32 ms, 3.46 m, 6.38 vvs, 6.89 s, 7.03 s, 7.22 s, 7.40 vs, 7.77 vs, 8.05 vs, 8.73 s, 8.90 s, 9.33 s, 9.71 s, 10.00 s, 10.97 vs, 12.02 s, 12.22 ms, 13.00 s, 14.46 s. The peak at 6.38 $\mu$ corresponds to the asymmetric $NO_2$ stretching vibration in compounds containing the $-CH_2NO_2$ group.

**Vapor–Liquid Partition Chromatography.**—A Perkin–Elmer, Model 154, vapor fractometer was used.

**Infrared Spectra.**—A Perkin–Elmer Infracord, Model 137, was used. The individual spectrograms were calibrated immediately after they were run using a polystyrene film as a standard. The absorption wave lengths are believed accurate to $\pm 0.02$ $\mu$.

**Ultraviolet Spectra.**—A Beckman ratio recording spectrophotometer, Model DK-2, was used. The wave length accuracy was checked by means of the mercury-in-quartz arc lamp.

**Mass Spectra.**—A Bendix Time-of-Flight spectrometer (Model 12) at an ionizing potential of 70 volts was used.

**Acknowledgment.**—This work was supported in part by the United States Air Force. We wish to thank Dr. Arnold Fainberg for chromatographic and infrared spectroscopic work, Dr. J. G. Smith, Jr., for the mass spectra determinations, and Mr. Howard Francis for elemental analyses and ultraviolet spectra.

(12) P. Tarrant and H. C. Brown, *J. Am. Chem. Soc.*, **73**, 1781 (1951).

# The Synthesis of DL-*threo*- and -*erythro*-Amicetose 2,4-Dinitrophenylhydrazones[1]

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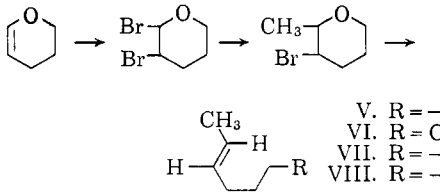
The DL-*erythro* (III) and -*threo* (IV) 2,4-dinitrophenylhydrazones of amicetose have been prepared from 1-nitro-4-hexene (VIII) which was synthesized from *trans*-4-hexen-1-ol (V). The structure proof of these isomers involved reaction with one mole of periodate. The natural isomer (I) was shown to be in the *erythro* series by an analysis of the rate of oxidation with periodate. Paper electrophoresis studies confirmed this result.

The antibiotic amicetin has been isolated from *Streptomyces plicatus*[3] and *Streptomyces vinaceus-drappus*.[4] Methanolysis of amicetin yielded, besides cytimidine,[5,6] a basic amino sugar, amosamine, and a neutral sugar amicetose isolated as its methyl glycoside in this laboratory.[7] Hydrolysis of the methyl glycoside of amicetose with 3 *N* hydrochloric acid gave free amicetose which was characterized as a crystalline 2,4-dinitrophenylhydrazone (I), m.p. 152–153°. The structure was established[7] by periodate cleavage of the 2,4-dinitrophenylhydrazone (I), which consumed only one mole of reagent in fifteen minutes with no further significant uptake in three hours. Acetaldehyde was isolated as its 2,4-dinitrophenylhydrazone in 51% yield as the volatile reaction product. The nonvolatile residue gave succindialdehyde bis-2,4-dinitrophenylhydrazone (II) in quantitative yield.

In this paper the synthesis of the DL-*erythro* and -*threo* isomers (III and IV) of amicetose 2,4-dinitrophenylhydrazone is reported and evidence presented that shows natural amicetose 2,4-dinitrophenylhydrazone to be in the *erythro* series.

4-Hexen-1-ol (V) was prepared from 3-bromo-2-methyltetrahydropyrane by the procedure of Brandon, Derfer, and Boord[8] in 55% over-all yield. The infrared absorption spectrum of (V) showed a strong absorption at 10.38 $\mu$, characteristic of a *trans* double bond. The 3,5-dinitrobenzoate derivative (VI) was prepared from V in 41% yield and also showed a strong absorption at 10.38 $\mu$. Addition of phosphorus tribromide to a cooled ethereal solution of *trans*-4-hexen-1-ol (V) gave a 53% yield of 1-bromo-4-hexene (VII). Treatment of VII with sodium nitrite in dimethylformamide[9] at −10° converted it to 1-nitro-4-hexene (VIII) in 65% yield.



V. R = −OH
VI. R = $C_6H_3(NO_2)_2$−COO−
VII. R = −Br
VIII. R = $-NO_2$

The reaction of 1-nitro-4-hexene (VIII) with silver acetate and iodine in acetic acid and water (1.5 moles)[10] gave the expected monoacetate of DL-1-nitro-4,5-

(1) This investigation was made possible by research grants CY 3772 and A 769 from the National Institutes of Health, Public Health Service.

(2) Wellcome Trust Travel Grant Recipient, 1960.

(3) T. H. Haskell, A. Ryder, R. P. Frohardt, S. A. Fusari, T. L. Jakubowski, and Q. R. Bartz, *J. Am. Chem. Soc.*, **80**, 743 (1958).

(4) J. W. Hinman, E. L. Caron, and C. DeBoer, *ibid.*, **75**, 5864 (1953).

(5) E. H. Flynn, J. W. Hinman, E. L. Caron, and D. O. Woolf, *ibid.*, **75**, 5867 (1953).

(6) T. H. Haskell, *ibid.*, **80**, 747 (1958).

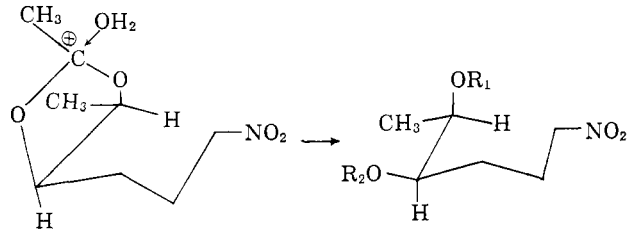
(7) C. L. Stevens, K. Nagarajan, and T. H. Haskell, *J. Org. Chem.*, **27**, 2001 (1962).

(8) R. C. Brandon, J. M. Derfer, and C. E. Booard, *J. Am. Chem. Soc.*, **72**, 2120 (1950); L. Crombie and S. H. Harper, *J. Chem. Soc.*, 1707 (1950), prepared V from the corresponding chlorotetrahydropyrane and showed the product to be mostly the *trans* isomer.

(9) N. Kornblum, H. O. Larsen, R. K. Blackwood, D. D. Mooberry, E. P. Oliveto, and G. E. Graham, *ibid.*, **78**, 1497 (1956).

(10) R. B. Woodward and F. V. Brutcher, *ibid.*, **80**, 209 (1958); S. Winstein and R. E. Buckles, *ibid.*, **64**, 2780, 2787 (1942).

hexanediol (IX) in 38.5% yield. In addition, the diacetate X was isolated in 14.5% yield. The monoacetate IX readily dissolved in 2 *N* aqueous potassium hydroxide solution containing a trace of methanol. The resulting *aci* salt solution was added to an 8 *N* sulfuric acid solution containing 2,4-dinitrophenylhydrazine reagent. Extensive alumina chromatography of the Nef reaction[11] products afforded a solid 2,4-dinitrophenylhydrazone, m.p. 105–106°, in 11% yield. Since this was the only isomer isolated, it was considered to be the DL-*threo* isomer IV of amicetose arising from an attack by water molecules on the reaction intermediate shown below.[10]



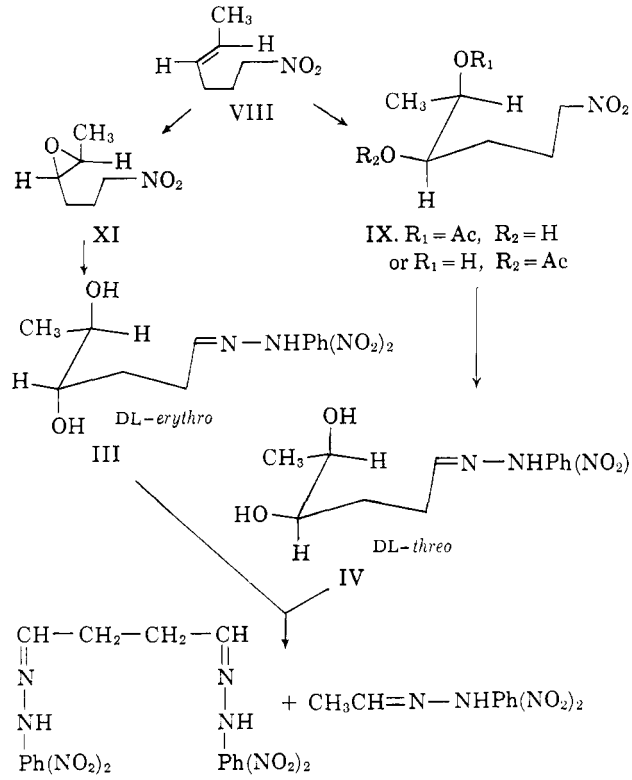
IX. $R_1$ = Ac, $R_2$ = H
or $R_1$ = H, $R_2$ = Ac
X. $R_1$ = $R_2$ = Ac

On paper chromatography the *threo* isomer IV ran as a single spot in several different systems. The $R_f$ values were identical to that of the natural isomer I. The ultraviolet and infrared spectra were very similar to those of natural isomer but the latter showed a slightly different pattern in the C—O stretching region (8.5 to 9.5 $\mu$). The *threo* isomer IV was further characterized as its diacetate IVa, m.p. 124.5–125.5°. This DL-diacetate also strongly resembled the optically active natural isomer diacetate in its paper chromatographic behavior and in its infrared and ultraviolet absorption spectral properties. 1-Nitro-4,5-hexanediol diacetate (X) was likewise converted to the *aci* salt and then treated under the conditions of the Nef reaction to give the same steric isomer IV, which was isolated as its 2,4-dinitrophenylhydrazone in 9% yield. Thus, both the monoacetate and the diacetate from the original hydroxylation reaction had the same steric configuration. The diacetate undoubtedly arose from the monoacetate by acetylation with the solvent acetic acid, as shown by Lucas, Mitchell, and Garner.[12]

Synthesis of the DL-*erythro* isomer (III) was attempted by the reaction of 1-nitro-4-hexene (VIII) with dry silver acetate and iodine in benzene.[13] However, in the reaction, an inseparable mixture of the DL-*threo* (IV) and *-erythro* (III) isomers was obtained (probably due to the presence of some water in the system). Hence, an alternate approach was sought.

Epoxidation of 1-nitro-4-hexene (VIII) with peracetic acid in chloroform gave 1-nitro-4,5-epoxyhexane (XI). This compound formed a water soluble sodium *aci* salt on addition to an aqueous solution of 1 *N* sodium hydroxide containing a trace of methanol. This *aci* salt solution was added slowly to a well stirred aqueous 8 *N* sulfuric acid 2,4-dinitrophenylhydrazine solution at 0°. Chromatography of the Nef reaction products over alumina gave a solid, m.p. 138°, in 3% yield, which had correct analysis for the DL-*erythro* isomer as would be expected from a *trans* opening of the epoxide by acid. Paper chromatography showed a single spot with an $R_f$ value identical with that of the natural isomer, and the ultraviolet and infrared absorption spectra closely resembled those of the natural isomer (I).

The structural identity of the DL-*erythro* (III) and *-threo* (IV)-isomers was proved by cleavage with sodium periodate. In each case one mole of periodate[14] was consumed. The periodate cleavate products were isolated as their 2,4-dinitrophenylhydrazones. Both the DL-*erythro* and *-threo* isomers gave succindialdehyde and acetaldehyde in good yields.



A comparative study of the rates of periodate uptake of the natural (I) and the DL-*threo* (IV) and *-erythro* (III) isomers was conducted in dilute solutions at 8°. The results (Table I) show that the DL-*threo* isomer (IV) consumed periodate more rapidly than either the DL-*erythro* (III) or natural (I) isomer, the latter two having identical rates within experimental error. This result indicated that the natural isomer was in the *erythro* series.

Confirmation of this result came from paper electrophoresis studies. Frahn and Mills[15] were able to separate *meso* and DL-*diols* by paper electrophoresis using a borate buffer. In the first run the three isomers (I, III, and IV), as their 2,4-dinitrophenylhydrazones, were dissolved in borax solution and then spotted as yellow zones on paper strips. However, on applying a potential each of the isomers was observed to move the same distance[16] within experimental error. In the

(11) W. E. Noland, *Chem. Rev.*, **55**, 137 (1955).

(12) H. J. Lucas, F. W. Mitchell, Jr., and H. K. Garner, *J. Am. Chem. Soc.*, **72**, 2138 (1950). Also see S. Winstein and R. E. Buckles, ref. 10.

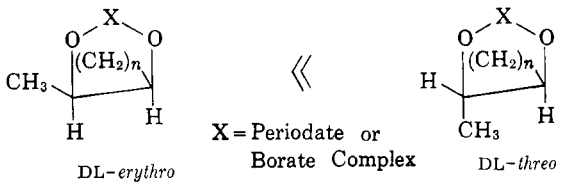
(13) C. Prevost, *Compt. rend.*, **196**, 1129 (1933); **197**, 1661 (1933); and C. Prevost and Wiemann, *ibid.*, **204**, 700 (1937).

(14) Stevens, Nagarajan, and Haskell[7] have previously shown in a control experiment that acetone 2,4-dinitrophenylhydrazone consumed a negligible amount of periodate even after 24 hours.

(15) J. L. Frahn and J. A. Mills, *Chem. Ind.* (London), 578 (1956).

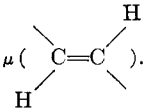
(16) Under the same experimental conditions heptaldehyde 2,4-dinitrophenylhydrazone did not move.

next run the three isomers (I, III, and IV) were dissolved in methanol and spotted as a yellow zone on paper strips. Paper electrophoresis was conducted in 0.083 *M* borax at a pH of 9.2 and a constant voltage of 380. Under these conditions, only the *threo* isomer (IV) was observed to migrate. This result was interpreted as evidence that the natural isomer (I) was in the *erythro* series. The results from the periodate and electrophoresis studies were not unexpected. Both reactions involve a *cis* cyclization between the diol group and either sodium periodate or sodium borate. In both cases a *cis* cyclization reaction of the *erythro* isomer (III) would involve methylalkyl chain eclipsing with an unfavorable conformation. A *cis* cyclization reaction of the *threo* isomer (IV) would involve a much less severe eclipsing (methyl and hydrogen) and was therefore expected to be favored. Thus, the *erythro* isomer (III) could be expected to consume periodate less rapidly and complex with borate and consequently migrate more slowly than the *threo* isomer (IV).




DL-*erythro* DL-*threo*

## Experimental

**The 3,5-Dinitrobenzoate of *trans*-4-Hexen-1-ol.**—A solution of 3,5-dinitrobenzoyl chloride (3.2 g., 13.8 mmoles) in dry benzene (10 ml.) was added at 0° to a solution of *trans*-4-hexen-1-ol (1.0 g., 10 mmoles), [prepared in 62% yield by the procedure of Brandon, Derfer, and Boord[8] from 3-bromo-2-methyltetrahydropyran (130.0 g., 0.72 mole) and sodium (32.5 g., 1.41 g.-atoms)], dry benzene (50 ml.), and pyridine (0.94 g.) and set aside at 25° for 12 hr. Pyridine hydrochloride was filtered and the benzene layer was washed consecutively with aqueous sodium carbonate and water. Evaporation of the solution after drying with anhydrous magnesium sulfate gave a pasty solid which was chromatographed in benzene over alumina to give the 3,5-dinitrobenzoate of *trans*-4-hexen-1-ol (1.2 g., 40.8%), m.p. 38.8–39.4° (recrystallized from methanol). It showed an infrared absorption at 10.38 $\mu$ (  ).

*Anal.* Calcd. for $C_{13}H_{14}N_2O_6$: C, 53.09; H, 4.80; N, 9.50 O, 32.63. Found: C, 53.25; H, 4.67; N, 9.46; O, 32.33.

**1-Bromo-4-hexene.**—Phosphorus tribromide (350.0 g., 1.3 moles) in dry ether (500 ml.) was added dropwise over a 2-hr. period to a solution of 4-hexen-1-ol (338.0 g., 3.38 moles) in dry ether (1 l.) and pyridine (30 ml.) in a bath cooled to −30°. The temperature was maintained at 20° for 24 hr. and then the reaction mixture was poured into ice–water (2 l.) and extracted with ether (5 × 100 ml.). The ether was washed with dilute aqueous sodium bicarbonate at 5°, water and then saturated aqueous sodium chloride. After drying the resulting solution over anhydrous magnesium sulfate, distillation gave an oil, 1-bromo-4-hexene (295.0 g., 53%), b.p. 63–65° (35 mm.), $n^{25}D$ 1.4652, infrared, 10.35 $\mu$.

*Anal.* Calcd. for $C_6H_{11}Br$: C, 44.18; H, 6.81. Found: C, 44.08; H, 6.81.

**1-Nitro-4-hexene.**—To a vigorously stirred solution of sodium nitrite (10.5 g., 0.15 mole) in dry dimethylformamide (500 ml.) cooled to −10° was added 1-bromo-4-hexene (16.3 g., 0.1 mole). After the addition, the temperature was allowed to rise to 20°, and after 4 hr. the solution was poured into ice-water (500 ml.). After extraction with petroleum ether, the organic layer was washed successively with water and saturated aqueous sodium chloride and then dried over anhydrous magnesium sulfate. Distillation gave two fractions; the lower boiling one had b.p. 53–55° (32.0 mm.), $n^{26}D$ 1.4224, infrared, 6.2 $\mu$, and amounted to 1.3 g. (probably a nitrite ester); and the higher boiling 1-nitro-4-hexene (7.5 g., 65%) which distilled as a colorless oil had b.p. 34–37° (1.2 mm.), $n^{25}D$ 1.4423, infrared ($CCl_4$), 6.45 $\mu$ (nitro) and 10.35 $\mu$.

*Anal.* Calcd. for $C_6H_{11}NO_2$: C, 55.79; H, 8.58; N, 10.85. Found: C, 55.49; H, 8.58; N, 11.09.

**The Mono- and Diacetates of 1-Nitro-4,5-hexanediol.**—To a well-stirred yellow complex of silver acetate (6.72 g., 0.04 mole) and iodine (5.20 g., 0.04 mole) in glacial acetic acid (240 ml.) maintained at 40° was added 1-nitro-4-hexene (2.58 g., 0.022 mole) in glacial acetic acid (10 ml.) and water (0.5 ml.). The mixture was stirred and heated under reflux for 16 hr., and the acetic acid was distilled *in vacuo* to yield a viscous oily residue. This oil was dissolved in benzene, filtered to remove insoluble inorganic material and after evaporation of the benzene gave a residual oil which on distillation afforded unchanged 1-nitro-4-hexene (0.31 g., 9.6%), b.p. 25° (0.05 mm.). The viscous residue was chromatographed over alumina to give on elution with petroleum ether DL-*threo*-1-nitro-4,5-hexanediol diacetate (0.9 g., 14.2%) as an oil, infrared, 5.75 $\mu$ (-OAc) and 6.45 $\mu$ (-nitro).

*Anal.* Calcd. for $C_{10}H_{17}NO_6$: C, 48.56; H, 6.93; N, 5.69; $CH_3CO$, 34.81. Found: C, 48.87; H, 6.87; N, 6.17; $CH_3CO$, 35.09.

Further elution of the original alumina column with benzene–ether (9:1) gave as a gum the monoacetate of DL-*threo*-nitro-4,5-hexanediol (1.44 g., 38.5%), infrared, 5.75 $\mu$ (-OAc) and 6.45 $\mu$ (nitro).

*Anal.* Calcd. for $C_8H_{15}NO_5$: C, 46.82; H, 7.40; N, 6.83; $CH_3CO$, 20.96. Found: C, 46.87; H, 7.10; N, 6.91; $CH_3CO$, 21.64.

**1-Nitro-4,5-epoxyhexane.**—To peracetic acid (3.5 g., 0.053 mole) in chloroform (233 ml.) cooled to 0° was added 1-nitro-4-hexene (5.4 g., 0.041 mole) in chloroform (10 ml.) and the mixture was set aside at 25° for 15 hr. After washing with aqueous sodium carbonate the chloroform layer was dried over anhydrous magnesium sulfate and concentrated to give 5.3 g. (82%) of an oil which was chromatographed over alumina. Elution with petroleum ether–ether (99:1) gave as an analytical sample a colorless oil, 1-nitro-4,5-epoxyhexane, b.p. 51–53° (0.1 mm.), $n^{25}D$ 1.4429, infrared, 6.45 $\mu$ (nitro).

*Anal.* Calcd. for $C_6H_{11}NO_3$: C, 49.63; H, 7.63; O, 33.06. Found: C, 49.53; H, 7.85; O, 33.05.

**DL-*threo*-Amicetose 2,4-Dinitrophenylhydrazone.**—DL-*threo*-1-Nitro-4,5-hexanediol monoacetate (1.44 g., 7 mmoles) was added slowly with stirring to a 2 *N* aqueous potassium hydroxide solution (15 ml.) containing methanol (1 ml.). The resultant solution was allowed to stand for 24 hr. and then added slowly with stirring to an aqueous 8 *N* sulfuric acid solution of 2,4-dinitrophenylhydrazine (1.8 g.) at −10°. The solution was allowed to stand for 1 hr. at −10°, diluted with water (1:1), extracted with chloroform, and the organic layer washed successively with water, with saturated sodium chloride, and then dried over anhydrous magnesium sulfate. Removal of chloroform gave a red gum which was chromatographed over alumina. Elution with chloroform afforded various compounds not further investigated. Elution with chloroform–methanol (98:2) gave 235 mg. (11%) of 2,3,6-trideoxy-DL-*threo*-hexose 2,4-dinitrophenylhydrazone (DL-*threo*-amicetose 2,4-dinitrophenylhydrazone) as yellow plates crystallized from chloroform, m.p. 105–106°, infrared (KBr), 2.95 $\mu$ (OH weak), 3.05 $\mu$ (-NH), 6.15 $\mu$ (C=N). $\lambda_{max}^{EtOH}$ 226 m$\mu$ ($\epsilon$ 14,620) and 356 m$\mu$ ($\epsilon$ 20,980). On paper chromatography, the $R_f$ value was 0.79 in a system of methanol saturated with heptane.

*Anal.* Calcd. for $C_{12}H_{16}N_4O_6$: C, 46.14; H, 5.16; N, 17.96; O, 30.76. Found: C, 46.06; H, 5.34; N, 17.93; O, 31.07.

Acetylation of the DL-*threo*-amicetose 2,4-dinitrophenylhydrazone (100 mg., 0.32 mmole) in acetic anhydride (2 ml.) and pyridine (2 ml.) at 40–45° for 16 hr. gave an orange gum after removal of the volatile reagents *in vacuo*. Chromatography over alumina gave on elution with pentane–ether (9:1) the diacetate of DL-*threo*-amicetose 2,4-dinitrophenylhydrazone (50 mg., 40%) which crystallized as yellow plates from ethanol, m.p. 127–127.5°, $\lambda_{max}^{EtOH}$ 226 m$\mu$ ($\epsilon$ 13,780) and 356 m$\mu$ ($\epsilon$ 20,490), infrared, 5.75, $\mu$ (-OAc).

*Anal.* Calcd. for $C_{16}H_{20}N_4O_6$: C, 48.49; H, 5.09; N, 14.33; O, 32.29. Found: C, 48.70; H, 5.26; N, 14.24; O, 32.33.

In a similar manner DL-*threo*-1-nitrohexanediol diacetate after formation of the *aci*-salt with aqueous potassium hydroxide

and subsequent Nef reaction with acidic 2,4-dinitrophenylhydrazine, gave a 9% yield of the DL-*threo*-amicetose 2,4-dinitrophenylhydrazone.

**DL-*erythro*-Amicetose 2,4-Dinitrophenylhydrazone.**—1-Nitro-4,5-epoxyhexane (4.7 g., 0.036 mole) was added slowly with stirring to 60 ml. of aqueous 1 *N* sodium hydroxide and the mixture was stirred for 2 hr. until it was homogeneous. This solution was added slowly with stirring over a period of 1 hr. to an aqueous solution of 2,4-dinitrophenylhydrazine in 9 *N* sulfuric acid (20 ml.) and methanol (50 ml.) cooled to 0°. The reaction mixture was maintained at 10° for 30 min., then diluted with an equal volume of water and set aside for a further 30 min. at 20°. After thorough extraction of the reaction mixture with chloroform, the extracts were washed with water, dried over anhydrous magnesium sulfate and the chloroform removed under reduced pressure to give a red gum which was chromatographed over alumina. On elution with chloroform various compounds were obtained which were not investigated further. Continued elution with chloroform–methanol (98:2) gave 2,3,6-trideoxy-DL-*erythro*-hexose 2,4-dinitrophenylhydrazone (DL-*erythro*-amicetose 2,4-dinitrophenylhydrazone). Recrystallization from chloroform gave 295 mg. (3%) of the pure isomer, m.p. 137.5–138°. Infrared (KBr), 2.96 $\mu$ (OH weak), 3.05 $\mu$ (-NH), 6.15 $\mu$ (C=N), $\lambda_{max}^{EtOH}$ 226 m$\mu$ ($\epsilon$ 13,700), 356 m$\mu$ ($\epsilon$ 20,000). Paper chromatography gave a spot with an $R_f$ value of 0.79 in a system of methanol saturated with heptane.

*Anal.* Calcd. for $C_{12}H_{16}N_4O_8$: C, 46.14; H, 5.16; N, 17.96; O, 30.76. Found: C, 46.34; H, 5.40; N, 18.16; O, 30.95.

**Product Isolation from Periodiate Cleavage of DL-*threo*- and -*erythro*-Amicetose 2,4-Dinitrophenylhydrazones.**—A solution of DL-*threo*-amicetose 2,4-dinitrophenylhydrazone (100 mg., 0.32 mmole), dioxane (2 ml.), and water (8 ml.) was added to 0.2 *N* aqueous sodium periodate (10 ml.) contained in a Claisen flask and set aside for 3 hr. The reaction mixture, after dilution with water (30 ml.), was steam distilled and the volatile products were passed through a trap at 0° containing 2,4-dinitrophenylhydrazine in 3 *N* hydrochloric acid. An orange precipitate was obtained which was extracted into benzene, after which the benzene solution was dried and passed over an alumina column. Elution with benzene gave acetaldehyde 2,4-dinitrophenylhydrazone (35 mg., 43%) as yellow needles, m.p. 163–165° after crystallization from ethanol. With an authentic sample, the mixture m.p. was undepressed and the infrared spectra were superimposable. Paper chromatography in a system of methanol saturated with heptane showed an $R_f$ value of 0.82 for the compound.

Excess ethanolic 2,4-dinitrophenylhydrazine-2 *N* hydrochloric acid reagent was added to the solution remaining in the flask after steam distillation. The resulting orange precipitate was filtered and recrystallized from hot dimethylformamide to give orange needles of succindialdehyde bis-2,4-dinitrophenylhydrazone (100 mg., 68%), m.p. 273–275°(d), $\lambda_{max}^{DMF}$ 580 m$\mu$ ($\epsilon$ 37,540), 458 m$\mu$ ($\epsilon$ 19,610), and 377 m$\mu$ ($\epsilon$ 18,060). In the usual paper chromatographic system of methanol saturated with heptane, the derivative had an $R_f$ value of 0.0 as did an authentic sample. With this authentic sample the mixture melting point was undepressed and the infrared spectra were superimposable.

In a similar manner the DL-*erythro*-2,4-dinitrophenylhydrazone was cleaved with sodium periodate to give both acetaldehyde identified as its 2,4-dinitrophenylhydrazone by m.p., infrared spectrum and paper chromatography and succindialdehyde bis-2,4-dinitrophenylhydrazone in 53%, identified in the same way.

Natural amicetose 2,4-dinitrophenylhydrazone has already been shown by Stevens, Nagarajan, and Haskell[7] to give, on cleavage with sodium periodate, both acetaldehyde 2,4-dinitrophenylhydrazone in 51% yield and succindialdehyde bis-2,4-dinitrophenylhydrazone quantitatively.

**The Rate of Periodate Cleavage.**—In a typical determination a sample (4.5 to 8.5 mg., 0.015 to 0.026 mmole) was dissolved in dioxane (5 ml.) and made up to a volume of 25 ml. in a graduate flask with water (15 ml.) and 0.02 *N* aqueous sodium periodate (5 ml., 0.05 mmole). The reaction mixture was allowed to stand at 8° and periodically an aliquot (2 ml.) was withdrawn by pipette and quenched by the addition of an aqueous solution of sodium bicarbonate and 0.01 *N* sodium arsenite (10 ml.). Then a few grains of potassium iodide were added and the solution was allowed to stand for a minimum period of 10 min. The solution was titrated with 0.01 *N* iodine to the first permanent blue color using starch as an indicator. Under the conditions of the experiment, it was necessary to back titrate and titrate again several times until a reproducible end point was obtained. In this manner the rates of oxidation of the 2,4-dinitrophenylhydrazones of DL-*threo*-, DL-*erythro*-, and natural amicetose were measured and compared (see Table I). A blank was run under identical conditions.

TABLE I

THE RATE OF PERIODATE CLEAVAGE AT 8 ± 2°

| Time, min. | Moles of periodate consumed: DL-*threo* (8.20 mg.) | DL-*erythro* (4.5 mg.) | Natural (8.14 mg.) |
|---|---|---|---|
| 7 | 0.70 | 0.10 | 0.20 |
| 18 | .70 | ... | .25 |
| 23 | ... | .26 | ... |
| 63 | ... | ... | .72 |
| 93 | .71 | .63 | ... |
| 190 | ... | ... | .91 |
| 218 | .79 | ... | ... |
| 240 | ... | .78 | ... |

**Paper Electrophoresis Studies.**—A methanolic solution of each of the 2,4-dinitrophenylhydrazones indicated in Table II was introduced onto a paper strip (Spinco no. 300-846, S and S 2043 A. gl.) as a narrow band and placed in a Beckman Spinco Model R paper electrophoresis system. A comparison was made using 0.083 *M* borax with a pH 9.2, at a constant voltage of 380 v. and a current of about 10–12 ma. After 2 hr. and 10 min., the strips were removed and dried. The migrations of the products are shown below in Table II.

TABLE II

| 2,4-Dinitrophenylhydrazone | Migration (cm.) |
|---|---|
| (a) Natural-amicetose | −0.1 |
| (b) DL-*erythro*-amicetose | −0.1 |
| (c) DL-*threo* amicetose | +2.3 |
| (d) Heptaldehyde | −0.05 |