The Synthesis of Positional Isomers of Muramic Acid[1]

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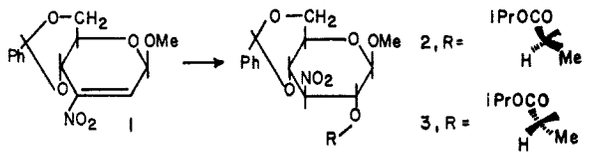
The synthesis of 3-amino-2-*O*-(L-1-carboxyethyl)-3-deoxy-D-glucose (**10**) and its D-1-carboxyethyl epimer (**17**) is reported. Nucleophilic addition to methyl 4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-β-D-*erythro*-hex-2-enopyranoside (**1**) of isopropyl L-lactate and isopropyl D-lactate, respectively, gave methyl 4,6-*O*-benzylidene-3-deoxy-2-*O*-[L-1-(isopropoxycarbonyl)ethyl]-3-nitro-β-D-glucopyranoside (**2**) and its D-1-(isopropoxycarbonyl)ethyl epimer (**3**) in yields of over 80%. The use of isopropyl DL-lactate afforded **2** and **3** in a ratio of 10:1. By standard methods of hydrolysis, catalytic hydrogenation, and acetylation, **2** and **3** were converted into **10** and **17** and various derivatives thereof, including the lactams **6** and **15**. A second synthesis started from methyl 3-acetamido-3-deoxy-β-D-glucopyranoside (**18**) whose 4,6-*O*-benzylidene derivative (**19**) was condensed with DL-2-chloropropionic acid and debenzylidenated to give methyl 3-acetamido-2-*O*-(L-1-carboxyethyl)-3-deoxy-β-D-glucopyranoside (**21**).

Muramic acid, a building unit of bacterial cell walls and spores, was first reported[2] in 1954 and was subsequently shown by degradation[3] and synthesis[4] to be 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose. The first synthesis[4] as well as later improvements[5,6,7] were based on condensations between derivatives of D-glucosamine and an α-halopropionic acid. For future structural and biochemical studies on microorganisms the availability of muramic acid isomers would appear desirable. We have therefore undertaken the synthesis of an epimeric pair of positional isomers of muramic acid, namely, the 3-amino-2-*O*-(L- and D-1-carboxyethyl)-3-deoxy-D-glucoses (**10** and **17**). First, both of these branched-chain sugars were obtained in a sequence of reactions beginning with a nitro olefin sugar which possessed the required nitrogen atom at C-3 and which, owing to its capability to undergo nucleophilic additions,[8] permitted the introduction of the side chain. Second, entry into one of the epimeric series was gained by the application, to a suitable derivative of 3-amino-3-deoxy-D-glucose, of the condensation with 2-chloropropionic acid that had been previously employed for the synthesis of muramic acid.[6,7]

Methyl 4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-β-D-*erythro*-hex-2-enopyranoside (**1**) was heated with isopropyl DL-lactate in benzene in the presence of a trace of potassium hydroxide. A mixture of epimeric addition products was formed from which pure methyl 4,6-*O*-benzylidene-3-deoxy-2-*O*-[L-1-(isopropoxycarbonyl)ethyl-3-nitro-β-D-glucopyranoside (**2**) and its D-1-(isopropoxycarbonyl)ethyl epimer (**3**) were obtained by fractional crystallization in yields of 56 and 5.5%, respectively. Separate reactions were performed under identical conditions with the optically active isopropyl L- and D-lactates in place of the racemate. They furnished the L and D adducts, respectively, in practically identical yields of about 83%. The 10:1 ratio of **2** and **3** isolated in the addition of the racemate could in part be due to steric factors favoring a faster reaction of the L ester. However, the difficulty of separating the more soluble **3** in pure condition from the epimeric mixture certainly contributed to the observed ratio.



Since the reactions leading to **2** and **3** did not involve any cleavage of bonds at the asymmetric carbon atoms in the isopropyl lactates, the latter unequivocally defined the side-chain configurations in the adducts. The *gluco* configuration of the hexose chain could *a priori* be assumed with some confidence, as similar nucleophilic additions to **1** had preferentially,[8] or exclusively,[9] led to *trans* diequatorial disposition of the substituents at C-2 and C-3. Definite proof was nonetheless deemed necessary and was obtained as shown in a subsequent paragraph.

The L-1-Carboxyethyl Series.—Because of the fact that from readily available, racemic isopropyl lactate the L adduct **2** was prepared rather more conveniently than the D adduct **3**, it was expedient to use the former for a more detailed pursuit of the planned path of synthesis.

Debenzylidenation of **2** furnished methyl 3-deoxy-2-*O*-[L-1-(isopropoxycarbonyl)ethyl]-3-nitro-β-D-glucopyranoside (**4**). This nitro glycoside, like others with related structures, exhibited the characteristic behavior in alkaline medium that has been the subject of a recent investigation.[10] In 0.01 *N* sodium hydroxide solution it showed an ultraviolet absorption peak at 255 mμ (ϵ_{max} 11,000) which is due to the nitronate ion. The peak disappeared on heating and, after 15 min at 98°, was replaced by a new peak at 298 mμ (ϵ_{max} 20,000). This change is attributed to the loss of a molecule of water resulting in the formation of a double bond between C-4 and C-5, in conjugation with the *aci*-nitro group.[10]

The platinum-catalyzed hydrogenation of **4** to the corresponding amine proved to be not quite straightforward, the product composition varying to some extent with the reaction conditions. Pressure (70 psi)

(1) This work was presented at the 153rd National Meeting of the American Chemical Society, Miami Beach, Fla., 1967. Abstract C 50. It was done as part of a Ph.D. Thesis to be submitted by F. K. at the University of Ottawa.

(2) C. S. Cummins and H. Harris, *Biochem. J.*, **57**, XXXII (1954); R. E. Strange and J. F. Powell, *ibid.*, **58**, 80 (1954).

(3) R. E. Strange and F. A. Dark, *Nature*, **177**, 185 (1956).

(4) R. E. Strange and L. H. Kent, *Biochem. J.*, **71**, 333 (1959).

(5) R. Lambert and F. Zilliken, *Ber.*, **93**, 2915 (1960); R. Gigg and P. M. Carroll, *Nature*, **191**, 495 (1961).

(6) Y. Matsushima and J. T. Park, *J. Org. Chem.*, **27**, 3581 (1962).

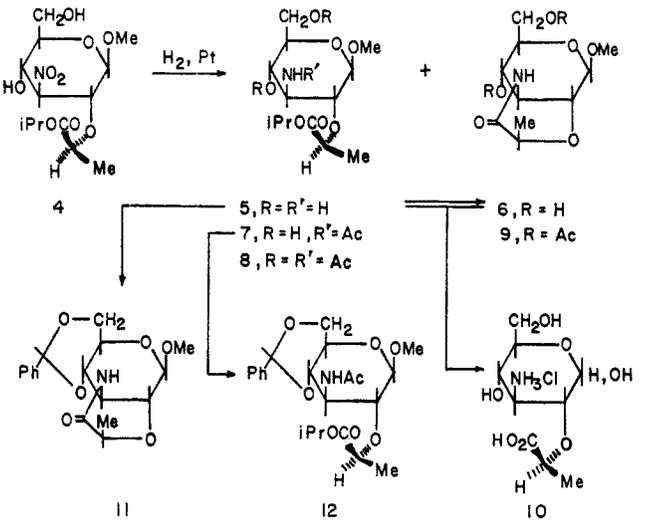
(7) H. M. Flowers and R. W. Jeanloz, *ibid.*, **28**, 1564, 2983 (1963); T. Osawa and R. W. Jeanloz, *ibid.*, **30**, 448 (1965).

(8) H. H. Baer and T. Neilson, *ibid.*, **32**, 1068 (1967).

(9) H. H. Baer, T. Neilson, and W. Rank, *Can. J. Chem.*, **45**, 991 (1967).

(10) H. H. Baer and F. Kienzle, *Ann.*, **695**, 192 (1966).

and a reaction time of 3–4 days were required, and even then a considerable quantity of unchanged starting material usually remained. This contrasts with the facile catalytic reductions that can normally be performed with nitro sugars. Hydrogenation in 2-propanol produced the expected amine, methyl 3-amino-3-deoxy-2-*O*-[L-1-(isopropoxycarbonyl)ethyl]-β-D-glucopyranoside (**5**), in a 29% yield, and in addition it gave about 10% of a crystalline by-product which has not yet been identified. When the hydrogenation was carried out in a 2-propanol–water mixture (1:1), only about 15% of the amine **5** and 9% of the by-product were isolated, but a third product was obtained in a 23% yield. The latter compound proved to be the lactam (**6**) of methyl 3-amino-3-deoxy-2-*O*-(L-1-carboxyethyl)-β-D-glucopyranoside; it obviously had arisen in a secondary reaction from the amino ester **5**. When an aqueous solution of crystalline **5** was heated on a steam bath for 3 hr, a nearly complete conversion into **6** occurred.[11]



The amino ester glycoside **5** was further characterized by preparing its *N*-acetyl derivative **7** and its *N*-acetyl-4,6-di-*O*-acetyl derivative **8**. The lactam **6** gave a 4,6-di-*O*-acetyl derivative (**9**). The spectral data of these compounds were in accord with the structures depicted. Thus, the infrared spectra[12] of the isopropyl esters **5** and **7** exhibited sharp carbonyl stretching bands at 1745 cm^{-1}. In the spectra of the acetates **8** and **9** these peaks occurred at 1745 and 1735 cm^{-1}, respectively, and they were broader because of the multiple ester groupings. No ester carbonyl absorption was present in **6**. The acetamido compounds **7** and **8** had amide-I bands at 1660 and amide-II bands at 1570–1560 cm^{-1}. The lactam diacetate **9** showed a sharp carbonyl stretching band at 1670 cm^{-1} which in the parent lactam **6** was shifted to 1640 cm^{-1} and broadened owing to hydrogen bonding. Neither lactam gave an amide-II band. The nmr spectrum[13] of the triacetyl compound **8** showed two sharp singlets corresponding to six and to three protons, at τ 7.95 and 8.05, which could be assigned to the two acetoxy groups and an equatorial acetamido group, respectively. The methoxyl resonance was a singlet at τ 6.57, and the *C*-methyl group of the lactic acid moiety gave a doublet centered at τ 8.63 and split by 7 cps. In the lactam diacetate **9** the acetoxy resonances appeared as separate singlets at τ 7.87 and 7.93; the methoxyl signal was at τ 6.46, and the *C*-methyl group gave a doublet at τ 8.53 with a splitting of 7 cps.

Acid hydrolysis of the amino ester glycoside **5** produced 3-amino-2-*O*-(L-1-carboxyethyl)-3-deoxy-D-glucose which crystallized as the hydrochloride **10**. The same reducing amino sugar was shown by paper chromatography[14,15] to arise from the lactam **6** by acid hydrolysis.

Treatment of the amino ester glycoside **5** with benzaldehyde and anhydrous zinc chloride led to simultaneous *O*-benzylidenation and lactam formation, yielding the lactam (**11**) of methyl 3-amino-4,6-*O*-benzylidene-2-*O*-(L-1-carboxyethyl)-3-deoxy-β-D-glucopyranoside, whereas similar benzylidenation of the *N*-acetyl derivative **7** proceeded without lactamization and afforded methyl 3-acetamido-4,6-*O*-benzylidene-3-deoxy-2-*O*-[L-1-(isopropoxycarbonyl)ethyl]-β-D-glucopyranoside (**12**). The preparation of the latter compound was undertaken because it provided a link with the second synthesis of muramic acid isomers and hence would be a key to the proof of the *gluco* configuration of its precursors.

The D-1-Carboxyethyl Series.—Debenzylidenation of the D-lactate adduct **3** gave methyl 3-deoxy-2-*O*-(D-1-(isopropoxycarbonyl)ethyl-3-nitro-β-D-glucopyranoside (**13**). The catalytic hydrogenation of **13** in 2-propanol was difficult like that of its L epimer **4**, and no crystalline product could be isolated in this case. However, the syrup obtained gave only one ninhydrin-positive spot on paper chromatograms,[14] and, since the R_{GN} value (2.0) was very close to that of the amine **5** (R_{GN} 1.9), it was concluded that its D epimer **14** was present. Hydrogenation of **13** in 2-propanol–water (1:1) afforded in a 45% yield the crystalline lactam **15** of methyl 3-amino-2-*O*-(D-1-carboxyethyl)-3-deoxy-β-D-glucopyranoside, which was characterized further by its 4,6-di-*O*-acetyl derivative (**16**). In the infrared, **15** showed a strong carbonyl stretching band at 1645 cm^{-1} as well as sharp OH bands in the 3600–3200 cm^{-1} region. There was no ester carbonyl, nitro, or amide-II absorption. The diacetate **16** lacked hydroxyl absorption and gave a weak band at 3230 (NH) as well as strong peaks at 1740 (ester carbonyl) and 1665 cm^{-1} (amide carbonyl). In the region from 1400 to 700 cm^{-1} both compounds exhibited the same general pattern as their corresponding epimers **6** and **9**, though with distinct differences in band positions and

(11) The isolation of **6** in the hydrogenation of **4** also offered a clue to the nature of the by-product mentioned above. The latter's analytical data lay between those of **6** and of the corresponding free amino acid, and also the infrared spectrum, melting point, and behavior in an acetylation (see Experimental Section) suggested that the by-product was a mixture of the two. A negative ninhydrin test does not necessarily rule out this possibility, since the amino acid might well be converted rapidly into the lactam under the test conditions.

(12) All infrared spectra were recorded from Nujol mulls on a Perkin-Elmer Infracord instrument.

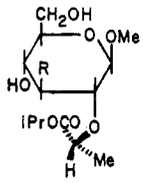
(13) All nmr spectra (60 Mcps) were obtained from deuteriochloroform solutions on a Varian HA-60 instrument.

(14) Paper chromatography was carried out by the descending technique on Whatman No. 1 paper in the system, pyridine–ethyl acetate–water–acetic acid (5:5:3:1), according to F. G. Fischer and H. Dörfel, *Z. Physiol. Chem.*, **301**, 224 (1955). The spots were indicated by a ninhydrin spray. R_{GN} = speed relative to glucosamine hydrochloride.

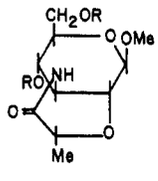
(15) The R_{GN} value was 0.8. There was an additional, faint spot of R_{GN} 1.25, which became more intense when the sample was heated *in vacuo* for 8 hr at 80° prior to chromatography. Perhaps the faster moving component was a lactone of **10**.

intensities. In the nmr spectrum of **16** the methoxyl and two *O*-acetyl signals appeared at τ 6.44, 7.89, and 7.94, respectively, and the *C*-methyl group gave a doublet centered at τ 8.55 with a splitting of 7 cps.

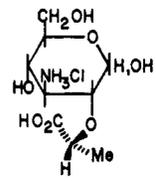
Both the crystalline lactam **15** and the syrup containing the amino ester glycoside **14** were hydrolyzed by hydrochloric acid. Although the expected reducing sugar, 3-amino-2-*O*-(D-1-carboxyethyl)-3-deoxy-D-glucose (**17**), was not isolated from the hydrolysis solutions, its presence was indicated by paper chromatography.[14] Like the crystalline L epimer **10**[15] the hydrolyzates presumed to contain **17** gave two ninhydrin spots (R_{GN} 0.8 and 1.3), but in the present case the faster spot was much stronger than the slower one.



13, R = NO_2
14, R = NH_2



15, R = H
16, R = Ac



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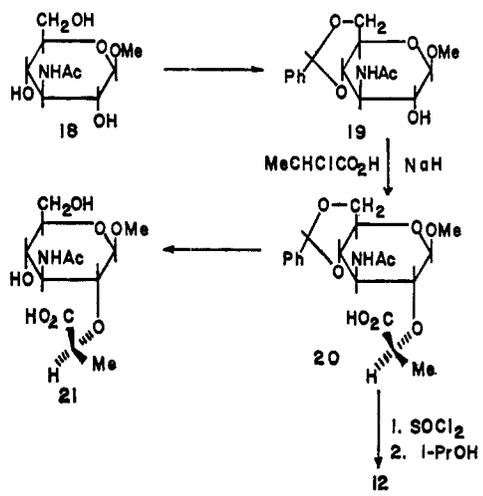
The second entry into the series of 2-*O*-carboxyethyl derivatives of 3-amino-3-deoxy-D-glucose was patterned after the syntheses[4-7] of muramic acid. The starting point was known methyl 3-acetamido-3-deoxy-β-D-glucopyranoside (**18**), which was blocked in positions 4 and 6 by benzylidenation. The benzylidene derivative **19** was to be condensed with isopropyl DL-2-chloropropionate in the hope that the 2-*O*-[L-1-(isopropoxycarbonyl)ethyl] derivative (**12**) or its D epimer (or both) would be obtained. This seemed to be the most convenient way of ascertaining by direct comparison the *gluco* configuration of the products from the first synthesis. Unfortunately, no condensation took place under the conditions specified.[16]

However, when DL-2-chloropropionic acid and **19** were refluxed in dioxane in the presence of excess sodium hydride, a yield of 30% of methyl 3-acetamido-



4,6-*O*-benzylidene-2-*O*-(L-1-carboxyethyl)-3-deoxy-β-D-glucopyranoside (**20**) was obtained. No epimer could be isolated. The acid **20** was then converted into its isopropyl ester by treatment with thionyl chloride followed by 2-propanol, and the ester so prepared proved to be identical with **12**. Finally debenzylidenation of **20**, which was effected by heating in water, led to methyl 3-acetamido-2-*O*-(L-1-carboxyethyl)-3-deoxy-β-D-glucopyranoside (**21**).

(16) In the syntheses of muramic acid, condensations in the position 3 of analogous glucosamine derivatives were achieved with ethyl 2-iodopropionate[4] and ethyl 2-bromopropionate.[5]

Experimental Section[12,14,17]

Methyl 4,6-*O*-Benzylidene-3-deoxy-2-*O*-[L-1-(isopropoxycarbonyl)ethyl]-3-nitro-β-D-glucopyranoside (2) and Methyl 4,6-*O*-Benzylidene-3-deoxy-2-*O*-[D-1-(isopropoxycarbonyl)ethyl]-3-nitro-β-D-glucopyranoside (3). A. Addition of Isopropyl DL-Lactate to Nitro Olefin 1.—To a solution of methyl 4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-β-D-*erythro*-hex-2-enopyranoside (**1**)[18] (1 g) in dry benzene (30 ml) was added isopropyl DL-lactate[19] (1 ml) and a small chip (about 5 mg) of potassium hydroxide. The mixture was gently boiled under reflux for 20 min, after which time thin layer chromatography (solvent A)[17] indicated a complete conversion of **1** into a faster-moving product. The yellowish solution was cooled, stirred briefly at 0° with 5 g of dry cation exchange resin, Rexyn 101 (H^+), and was then treated with activated charcoal and evaporated to give a slightly yellowish syrup. Crystallization from ethyl acetate–petroleum ether in the course of several hours at 0° gave needles (550 mg) of the L adduct **2**, mp 124°. Upon recrystallization from aqueous ethanol it showed mp 126° and $[\alpha]^{23}D$ −69.7° (*c* 0.85 in ethanol).

Anal. Calcd for $C_{20}H_{27}NO_9$ (425.4): C, 56.40; H, 6.35; N, 3.29. Found: C, 56.68; H, 6.43; N, 3.30.

The mother liquor from the first crop of **2** was partially evaporated whereby a crystalline mixture (410 mg, mp 85–89°) of **2** and **3** was obtained. Recrystallization from aqueous ethanol yielded 200 mg of **2** (mp 124°), thus raising its total yield to 52%. From the mother liquor of this recrystallization a material melting at 80–81° was isolated which was recrystallized three more times from aqueous ethanol to show mp 96–97°. It was the D adduct **3**, $[\alpha]^{23}D$ −40.0° (*c* 1 in ethanol). The yield of pure **3** was 53 mg (3.6%). In a similar experiment, 1.50 g of **1** gave 1.223 g (56%) of **2** and 0.120 g (5.5%) of **3**.

Anal. Calcd for $C_{20}H_{27}NO_9$ (425.4): C, 56.40; H, 6.35; N, 3.29. Found: C, 56.52; H, 6.23; N, 3.50.

A mixture melting point of **2** and **3** was strongly depressed, and the infrared spectra[12] showed small but distinct differences in the region of 1100–800 cm^{-1}. Both epimers had their ester carbonyl bands at 1750 cm^{-1}; the asymmetrical nitro vibration was at 1560 in **2** and at 1565 cm^{-1} in **3**.

B. Addition of Isopropyl L-Lactate to Nitro Olefin 1.—Isopropyl L-lactate was prepared[19] from L(+)-lactic acid[20] and had $[\alpha]^{23}D$ −9.1° (*c* 2.5 in methanol). Its addition to nitro olefin **1** was performed as in A, 610 mg of **1** and 0.6 ml of ester being used. There was obtained 730 mg (82.5%) of crude **2** with mp 116–118° which was raised by recrystallization to 119° and was not depressed upon admixture of **2** from A, $[\alpha]^{23}D$ −63.5° (in ethanol). The identity of the samples was supported by the infrared spectra and by the debenzylidenations (see below) that led to identical products.

C. Addition of Isopropyl D-Lactate to Nitro Olefin 1.—For the preparation of isopropyl D-lactate, 5 g of the calcium salt (tetrahydrate) of D(−)-lactic acid[21] and 10 g of Rexyn-101 (H^+) were suspended in dry benzene (60 ml) and 2-propanol (15 ml), and the mixture was refluxed with magnetic stirring in an apparatus equipped with an automatic water separator. Refluxing was continued until no more water was collected in the separator. The filtered solution was then evaporated, and the ester distilled at 71–76° (22–24 mm), yield 2.5 g, $[\alpha]^{23}D$ +9.2° (*c* 2.5 in methanol).

Using the procedure of A, nitro olefin **1** (1.0 g) and isopropyl D-lactate (1 ml) were allowed to interact and gave 1.2 g (83%)

(17) Thin layer chromatography was performed on silica gel G plates, with (A) ethyl acetate–petroleum ether (bp 30–60°) (3:1) or (B) ethyl acetate–ethanol–petroleum ether (bp 30–60°) (2:1:1).

(18) H. H. Baer and T. Neilson, *Can. J. Chem.*, **43**, 840 (1965).

(19) A. I. Vogel, "A Textbook of Practical Organic Chemistry," 3rd ed, Longmans, Green and Co., Ltd., London, 1956, p 387.

(20) Obtained from CalBiochem, Inc., Bethesda, Md.

(21) Obtained from Sigma Chemical Co., St. Louis, Mo.

of **3**, mp 91–92°. Admixture of **3** from A caused no depression, and the infrared spectra were identical.

D. Reaction of Isopropyl L-Lactate with Methyl 2-*O*-Acetyl-4,6-*O*-benzylidene-3-deoxy-3-nitro-β-D-glucopyranoside.[22]—The acetyl derivative (350 mg) was added in small portions to isopropyl L-lactate (2 ml) containing sodium isopropoxide (250 mg) and 2-propanol (0.2 ml).[23] The crystals dissolved on swirling, gentle warming being required toward the end of the operation. The solution was kept at room temperature for 30 min and then mixed under ice cooling with ethanol (5 ml) containing acetic acid (2 ml). Dropwise addition of water gave a crystalline precipitate of crude **2** which was washed with ethanol–water; 380 mg, mp *ca.* 110°. Pure **2** (needles of mp 120–121°) was obtained only after three recrystallizations which reduced the yield to 105 mg and so offset partially the advantages of this simpler procedure over method A.

Methyl 3-Deoxy-2-*O*-(L-1-(isopropoxycarbonyl)ethyl]-3-nitro-β-D-glucopyranoside (4).—The benzylidene compound **2** (400 mg) was hydrolyzed by heating on a steam bath for 25 min, with 15 ml of 75% acetic acid. Complete debenzylidenation was revealed by thin layer chromatography (solvent A)[17] which showed the disappearance of fast-moving **2** and the sole presence of a slow-moving new product. The solvent was removed, and water was added twice to the residue and evaporated. The residue was then recrystallized from hot water. The product **4** (216 mg, 67%) had mp 148–149°, raised to 151° by another recrystallization, $[\alpha]^{23}D$ −27° (*c* 1 in ethanol). Infrared bands for the hydroxyl, ester carbonyl, and nitro groups were at 3480–3260, 1750, and 1555 cm^{-1}, respectively.

Anal. Calcd for $C_{13}H_{23}NO_9$ (337.3): C, 46.35; H, 6.83. Found: C, 46.36; H, 6.92.

Methyl 3-Deoxy-2-*O*-[D-1-(isopropoxycarbonyl)ethyl]-3-nitro-β-D-glucopyranoside (13).—Compound **13** was obtained from 100 mg of the benzylidene derivative **3** by the procedure described in this paper for **4**. However, the crude product was syrupy at first and crystallized, as long needles, from an ethanol solution by slow evaporation in the air. Upon recrystallization from water it melted at 102–103°, yield 46 mg (58%). Infrared bands for the hydroxyl, ester carbonyl, and nitro groups were at 3450–3250, 1753, and 1563 cm^{-1}, respectively.

Anal. Calcd for $C_{13}H_{23}NO_9$ (337.3): C, 46.35; H, 6.83; Found: C, 46.51; H, 7.06.

Hydrogenation of 4. A. In 2-Propanol.—The nitro glycoside **4** (400 mg) and platinum dioxide (100 mg) in 2-propanol (50 ml) were shaken for 4 days under hydrogen at 70 psi. Evaporation of the filtered solution gave a syrup that was dissolved in a small amount of ethyl acetate and kept at 4° for crystallization. After 3 days 105 mg (28.8%) of prisms of methyl 3-amino-3-deoxy-2-*O*-[L-1-(isopropoxycarbonyl)ethyl]-β-D-glucopyranoside (**5**) had been deposited, mp 183°, unchanged upon recrystallization from 2-propanol–ethyl acetate, $[\alpha]^{23}D$ −43.5° (*c* 1 in water), R_{GN} 1.9.[14]

The compound showed strong infrared absorption at 3450 (OH, NH) and 1745 (ester carbonyl), and a medium peak at 1585 cm^{-1} (NH bending).

Anal. Calcd for $C_{13}H_{25}NO_7$ (307.3): C, 50.80; H, 8.21; N, 4.56. Found: C, 50.65; H, 8.13; N, 4.51.

The mother liquor from **5** was concentrated a little, and petroleum ether was added to incipient turbidity. After several hours at 0° a *by-product* (44 mg) was collected, mp 134–137°. Recrystallization from the same solvents gave 32 mg of crystals melting at 150–151° (air dry), or at 165–166° with slight prior sintering (dried *in vacuo*). A mixture melting point with **4** was depressed, and a ninhydrin test was negative. The analytical data (Found: C, 47.36; H, 7.13; N, 5.18.) of this by-product lay between those expected for methyl 3-amino-2-*O*-(L-1-carboxyethyl)-3-deoxy-β-D-glucopyranoside (Calcd for $C_{10}H_{19}NO_7$: C, 45.28; H, 7.22; N, 5.28.) and for its lactam (**6**) (Calcd for $C_{10}H_{17}NO_6$: C, 48.65; H, 6.94; N, 5.67.). The infrared spectrum showed the same general features as that of **6**, but in addition there was a strong peak at 1710 cm^{-1} and weak absorption in the region of 2700–2400 cm^{-1}, which could be attributed to a free carboxyl group. A sample of the by-product (36 mg) was treated with acetic anhydride (0.25 ml) and pyridine (0.5 ml) for 7 hr at 23°. After two evaporations with excess toluene and two more with excess ethanol a white solid remained. It gave 17 mg of thin prisms, mp 193°, on recrystallization from absolute ethanol and petroleum ether. The infrared spectrum and analytical values (Found: C, 51.10; H, 6.70.) were very similar to those of the lactam diacetate (**9**).

B. In 2-Propanol–Water.—The nitro glycoside **4** (600 mg) and platinum dioxide (200 mg) in a mixture of water (25 ml) and 2-propanol (25 ml) were hydrogenated at 60 psi for 2 days. Work-up as described under A yielded 80 mg of amino glycoside **5**, mp 178–180°. The mother liquor was found to contain mainly starting material. It was brought to dryness, and the hydrogenation was repeated (3 days) using 100 mg of fresh catalyst and a pressure of 70 psi. Evaporation followed by crystallization of the residue from ethyl acetate furnished 53 mg of a substance which had mp 138–140° (raised to 151–152° by recrystallization from ethyl acetate–petroleum ether) and proved identical with the *by-product* described under A. The ethyl acetate solution from which the by-product had separated was concentrated, and addition of petroleum ether gave crystals (170 mg) melting at 142–146°. Recrystallization of this crop from ethyl acetate–petroleum ether afforded 100 mg of prisms, mp 166°, showing an infrared spectrum different from those of **4**, **5**, and the by-product. There was no peak attributable to ester or carboxyl carbonyl but a strong peak at 1640 cm^{-1} that was assigned to an amide grouping (see discussion). The compound was the lactam (**6**) of methyl 3-amino-2-*O*-(L-1-carboxyethyl)-3-deoxy-β-D-glucopyranoside, $[\alpha]^{23}D$ −73.5° (*c* 1.3 in methanol).

Anal. Calcd for $C_{10}H_{17}NO_6$ (247.3): C, 48.65; H, 6.94; N, 5.67. Found: C, 48.72; H, 7.06; N, 5.62.

A sample (16 mg) of the amino ester **5** in water (5 ml) was heated on a steam bath for 3 hr, with the addition of a small drop of acetic acid. Thin layer chromatography (solvent B)[17] indicated a nearly complete conversion of the slow-moving **5** into the fast-moving lactam **6**.

Lactam Diacetate (9).—A sample (40 mg) of lactam **6** was acetylated overnight at 23° with acetic anhydride (0.25 ml) and pyridine (0.5 ml). The reaction mixture was evaporated thrice with excess toluene and twice with ethanol. The colorless crystalline residue was dried *in vacuo* and had mp 191°. The yield was 53 mg (quantitative). Recrystallization from absolute ethanol gave needles (44 mg), and the melting point was unchanged. The product was the lactam (**9**) of methyl 4,6-di-*O*-acetyl-3-amino-2-*O*-(L-1-carboxyethyl)-3-deoxy-β-D-glucopyranoside. Strong infrared bands at 1735 and 1670 cm^{-1} indicated ester and amide carbonyl groups.

Anal. Calcd for $C_{14}H_{21}NO_8$ (331.3): C, 50.80; H, 6.36; N, 4.23. Found: C, 50.69; H, 6.41; N, 4.17.

Benzylidene Lactam (11).—A sample (70 mg) of amino ester **5**, anhydrous zinc chloride (200 mg), and benzaldehyde (2 ml) were stirred together for 4 hr at 23°. The reaction mixture was agitated thoroughly with water (5 ml) and petroleum ether (20 ml) in an ice–water bath. After 10 min, the white precipitate was filtered off, washed with cold water and with petroleum ether, and then immediately recrystallized from ethanol–water (7:3). The yield was 32 mg (42%) of the lactam (**11**) of methyl 3-amino-4,6-*O*-benzylidene-2-*O*-(L-1-carboxyethyl-3-deoxy-β-D-glucopyranoside; thin platelets of mp 257° remained unchanged on further recrystallization. The amide-I band was at 1663 cm^{-1}; there was no amide-II band.

Anal. Calcd for $C_{17}H_{21}NO_6$ (335.4): C, 60.95; H, 6.31; N, 4.18. Found: C, 60.79; H, 6.23; N, 4.28.

Methyl 3-Acetamido-3-deoxy-2-*O*-[L-1-(isopropoxycarbonyl)-ethyl]-β-D-glucopyranoside (7).—The amino ester **5** (85 mg) was dissolved in water (2 ml) and methanol (1 ml). Acetic anhydride (0.1 ml) was added with ice cooling, and the mixture was then stirred for 90 min at ambient temperature. Evaporation gave a white solid (**7**) which was recrystallized from 2-propanol, mp 189–191°, raised to 192–193° by a second recrystallization. The yield was 63 mg (65%). The infrared spectrum had several sharp bands in the region 3500–3150 (OH, NH), a band at 1745 (ester carbonyl), and amide-I and -II bands at 1660 and 1570 cm^{-1}.

Anal. Calcd for $C_{15}H_{27}NO_8$ (349.4): C, 51.55; H, 7.79; N, 4.01. Found: C, 51.47; H, 7.79; N, 4.10.

Methyl 3-Acetamido-4,6-di-*O*-acetyl-3-deoxy-2-*O*-[L-1-(isopropoxycarbonyl)ethyl]-β-D-glucopyranoside (8).—The amino ester **5** (70 mg) was acetylated, at 23° for 18 hr, with acetic anhydride (0.5 ml) and pyridine (1 ml). The reaction mixture

(22) This acetyl derivative is the starting compound for the preparation of the nitro olefin **1**.[18] It has been used previously[5] in elimination–addition reactions that led to the replacement of the acetoxy by alkoxy substituents, thus obviating the preparation of **1**.

(23) Omission of the 2-propanol resulted in the recovery of unchanged starting material and failure to produce **2**.

was worked up using the common chloroform extraction procedure. The triacetyl derivative **8** was obtained as a white microcrystalline powder, mp 148–149°, from ethyl acetate–petroleum ether, in a yield of 66 mg (67%). Infrared bands were at 3320 (NH stretching), 1745 (ester carbonyl), 1660 (amide-I), and 1560 cm^{-1} (amide-II).

Anal. Calcd for $C_{19}H_{31}NO_{10}$ (433.5): C, 52.75; H, 7.22; N, 3.24. Found: C, 52.96; H, 7.36; N, 3.42.

Methyl 3-Acetamido-4,6-*O*-benzylidene-3-deoxy-2-*O*-[L-1-(isopropoxycarbonyl)ethyl]-β-D-glucopyranoside (12) from 7.—The acetamido ester **7** (60 mg), anhydrous zinc chloride (200 mg), and benzaldehyde (1.5 ml) were stirred together for 5 hr at 23°. Work-up as described for **11** yielded 43 mg (57%) of long needles (from 90% ethanol), mp 226–227°, $[\alpha]^{23}D$ −61° (*c* 0.5 in ethanol). Infrared bands were at 3320 (NH stretching), 1748 (ester carbonyl), 1650 (amide-I), and 1560 cm^{-1} (amide-II).

3-Amino-2-*O*-(L-1-carboxyethyl)-3-deoxy-D-glucose Hydrochloride (10).—The amino glycoside **5** (150 mg) was hydrolyzed with 3 *N* hydrochloric acid (10 ml) at 100° for 5 hr. Most of the acid was removed by distillation *in vacuo*, and the remaining nearly colorless syrup was dissolved in water and brought to pH 5.2 by treatment with Dowex 1-X2(OH^-). Evaporation, ultimately with two additions of ethanol, left a white solid which was washed with ether. The yield was 103 mg (73.5%). The material reduced Fehling solution, showed $[\alpha]^{23}D$ +23° (*c* 1.5 in water) and R_{GN} 0.8.[14,15] It gradually decomposed on heating above 100°. A combustion residue revealed the presence of some inorganic impurity.

Anal. Calcd for $C_9H_{18}ClNO_7$ (287.7): C, 37.65; H, 6.31; N, 4.88. Found: C, 38.01; H, 6.91; N, 4.34.

The lactam **6** (2 mg) in 5 *N* hydrochloric acid (0.3 ml) was heated in a sealed tube at 110° for 5 hr. Subsequent paper chromatography gave a strong ninhydrin-positive spot of R_{GN} 0.80.

Hydrogenation of Nitro Glycoside 13. A. In 2-Propanol.—The nitro glycoside **13** (600 mg) was hydrogenated as described for **4**. A syrup was obtained which could not be crystallized from a variety of solvents. Paper chromatography[14] revealed the presence of one ninhydrin-positive compound, R_{GN} 2.0, presumed to be methyl 3-amino-2-*O*-[D-1-(isopropoxycarbonyl)-ethyl]-3-deoxy-β-D-glucopyranoside (**14**). When an approximately 1% solution of **14** in water containing a little acetic acid was heated on a steam bath for 2.5 hr, lactam **15** was produced although the lactamization was rather incomplete and other products were indicated by thin layer chromatography (solvent B)[17] to be present. Nevertheless, crystalline **15** was isolated in 7% yield and identified by its infrared spectrum with **15** obtained under B.

B. **In 2-Propanol–Water.**—The nitro glycoside **13** (320 mg) and platinum dioxide (100 mg) in a mixture of water (25 ml) and 2-propanol (25 ml) were hydrogenated at 54 psi for 3 days. The solution was treated with activated charcoal and evaporated to give a colorless syrup that crystallized in large prisms after standing at 25°. The material was triturated with ethyl acetate–2-propanol (3:1) at 0°, and it was then isolated after several hours and washed with chilled ethyl acetate, yielding 75 mg (45%) of the lactam (**15**) of methyl 3-amino-2-*O*-(D-1-carboxyethyl)-3-deoxy-β-D-glucopyranoside, mp 204–205° dec, $[\alpha]^{23}D$ −40.0° (*c* 0.6 in methanol). Infrared absorption was in the region of 3600–3200 (OH, several bands) and at 1645 cm^{-1} (amide carbonyl). There was no amide-II band. In the fingerprint region the spectrum was generally similar to that of **6**, though small differences were observed.

Anal. Calcd for $C_{10}H_{17}NO_6$ (247.3): C, 48.65; H, 6.94; N, 5.67. Found: C, 48.50; H, 7.04; N, 5.60.

The mother liquor appeared to contain mainly starting material, according to thin layer chromatography. A ninhydrin test was negative.

A sample (53 mg) of lactam **15** was acetylated as described for the preparation of **9** from **6**. Recrystallization from absolute ethanol of the product gave 41 mg of oblong platelets, mp 167–168°, the lactam (**16**) of methyl 4,6-di-*O*-acetyl-3-amino-2-*O*-(D-1-carboxyethyl)-3-deoxy-β-D-glucopyranoside. Infrared bands were at 1735 and 1675 cm^{-1}.

Anal. Calcd for $C_{14}H_{21}NO_8$ (331.3): C, 50.80; H, 6.36; N, 4.23. Found: C, 50.59; H, 6.33; N, 4.03.

Hydrolyses of **14** and **15** with 5 *N* hydrochloric acid in sealed tubes at 110° for 5 hr resulted in the formation of 3-amino-2-*O*-(D-1-carboxyethyl)-3-deoxy-D-glucose hydrochloride (**17**), as indicated by paper chromatography[14] in which both **14** and **15** gave the same pattern, R_{GN} 1.3 (strong) and 0.8 (weak) (compare also ref 15).

Methyl 3-Acetamido-4,6-*O*-benzylidene-3-deoxy-β-D-glucopyranoside (19).—Methyl 3-acetamido-3-deoxy-β-D-glucopyranoside (**18**)[24] (410 mg) was stirred with benzaldehyde (5 ml) and anhydrous zinc chloride (1 g) for 20 hr. The mixture was stirred vigorously, in an ice-bath, with 10 ml of water and 50 ml of petroleum ether. The precipitate was washed with the same solvents and immediately recrystallized from 90% ethanol, from which **19** crystallized as a monohydrate, mp 294°, $[\alpha]^{23}D$ −78° (*c* 0.8 in pyridine). The yield was 410 mg (69%). The infrared spectrum showed broad hydroxyl absorption (3600–3200 cm^{-1}) and a water peak (1615 cm^{-1}) that persisted during the drying for analysis (6 hr at 78° *in vacuo*). The amide-I and -II bands were at 1645 and 1555 cm^{-1}.

Anal. Calcd for $C_{16}H_{21}NO_6 \cdot H_2O$ (341.4): C, 56.55; H, 6.56; N, 4.37. Found: C, 56.40; H, 6.66; N, 4.21.

An anhydrous product was obtained by dissolution of the hydrate in absolute ethanol, evaporation of the solution, and several repetitions of this procedure. Recrystallized from dioxane–petroleum ether, it then melted at 296° dec and no longer showed the peak at 1615 cm^{-1}. Three sharp peaks were now at 3570, 3300, and 3120 cm^{-1}.

Methyl 3-Acetamido-4,6-*O*-benzylidene-2-*O*-(L-1-carboxyethyl)-3-deoxy-β-D-glucopyranoside (20).—Sodium hydride (550 mg) was added to a solution of *anhydrous* **19** (650 mg) in dry dioxane (100 ml). The mixture was gently refluxed for 1 hr and was then allowed to cool to 65°. DL-2-Chloropropionic acid (1.2 ml) and, after 1 hr, another portion (2 g) of sodium hydride were added, and the mixture was magnetically stirred for 19 hr at 65°. Upon cooling, 70 ml of water was cautiously introduced, and the solution was concentrated *in vacuo* to a small volume. The yellowish solution obtained on diluting the concentrate with water was extracted once with chloroform (which was discarded), and then it was acidified, under ice cooling, with 30 ml of chilled 6 *N* hydrochloric acid. Without delay the acidic solution was extracted four times with chloroform, and the combined extracts were immediately washed with four portions of cold water, dried briefly with sodium sulfate, and evaporated to give a solid residue. The residue was triturated with a few milliliters of hot methanol, and, after cooling, 262 mg (33%) of **20**, mp 282–283°, was collected. Recrystallization from methanol, with the addition of a little petroleum ether, furnished fine needles, mp 287°, $[\alpha]^{23}D$ −48° (*c* 0.9 in methanol). Infrared absorption was at 3300 (sharp, NH), 2700–2500 (weak, OH), 1705 (strong, CO_2H), 1655 (amide-I), and 1560 cm^{-1} (amide-II).

Anal. Calcd for $C_{19}H_{25}NO_8$ (395.4): C, 57.71; H, 6.37; N, 3.54. Found: C, 57.88; H, 6.58; N, 3.30.

From the mother liquor were isolated small fractions of crystals (26 and 85 mg) which appeared to be impure **20** and starting material **19**. The remainder was a brown syrup that may have contained the D-1-carboxyethyl isomer.

Methyl 3-Acetamido-4,6-*O*-benzylidene-3-deoxy-2-*O*-[L-1-(isopropoxycarbonyl)ethyl]-β-D-glucopyranoside (12) from 20.—One hundred milligrams of **20** was dissolved in 1 ml of thionyl chloride. After 5 min the excess chloride was quickly evaporated and the treatment was repeated. The solid residue was immediately dissolved in 2-propanol (5 ml), and the mixture was twice evaporated after addition of fresh 2-propanol. The isopropyl ester **12** (47 mg, mp 218–219°) crystallized from 50% aqueous ethanol, and upon recrystallization from 90% ethanol it showed mp 220–221° and $[\alpha]^{23}D$ −56° (*c* 0.5 in ethanol). A mixture melting point with **12**, obtained from **11**, was 220–221°, and the infrared spectra of both compounds were identical.

Methyl 3-Acetamido-2-*O*-(L-1-carboxyethyl)-3-deoxy-β-D-glucopyranoside (21).—A suspension of benzylidene compound **20** (70 mg) in water (15 ml) was heated on the steam bath for 2 hr, during which complete dissolution occurred. The solution was evaporated and the residue was once more heated, for 1 hr, in 10 ml of water. A smell of benzaldehyde was noticed. Evaporation gave a colorless syrup which, on two evaporations with ethanol and trituration with a small amount of ether, yielded fine needles (32 mg, 59%) of crude **21**, mp 172–175°. Three recrystallizations from ether containing 1 drop of methanol gave 21 mg of pure **21**, mp 190–191°, $[\alpha]^{23}D$ −4.8° (*c* 0.5 in methanol). Infrared absorption was in the 3600–3200- and 2700–

(24) H. H. Baer, *Ber.*, **93**, 2865 (1960); *J. Am. Chem. Soc.*, **83**, 1882 (1961).

2500-cm^{-1} regions (OH, NH), and at 1720 (CO_2H), 1635 (amide-I), and 1565 cm^{-1} (amide-II).

Anal. Calcd for $C_{12}H_{21}NO_8$ (307.3): C, 46.90; H, 6.89; N, 4.56. Found: C, 47.00; H, 7.02; N, 4.64.

Registry No.—2, 14001-79-7; 3, 13942-26-2; 4, 13942-32-0; 5, 14001-80-0; 6, 13942-28-4; 7, 14001-81-1; 8, 14038-36-9; 9, 13942-29-5; 10, 13942-30-8; 11, 13978-01-3; 12, 13942-31-9; 13, 13942-32-0; 15, 14001-82-2; 16, 14001-83-3; 17, 13942-33-1; 19, 4603-73-0; 20, 13942-35-3; 21, 13942-36-4.

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Rearrangments of 2-(Aminoalkylthio)-2-thiazolines and -5,6-dihydro-4H-1,3-thiazines

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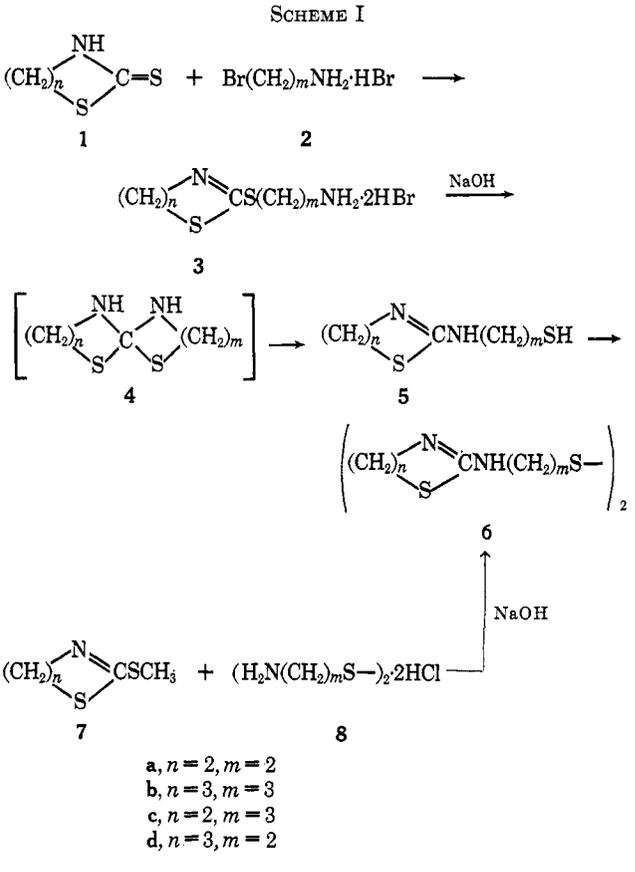
Treatment of 2-(3-aminopropylthio)-5,6-dihydro-4H-1,3-thiazine dihydrobromide (**3b**) with base yielded compound **9** by an intermolecular reaction, in addition to disulfide **6b** by rearrangement. The same amine dihydrobromide (**3c**), containing a thiazoline ring, was obtained from the aminopropylation of 2-thiazolidinethione and the aminoethylation of tetrahydro-2H-1,3-thiazine-2-thione. On rearrangement it afforded the disulfide with the five-membered heterocyclic ring, **6c**. The latter was also obtained both from the reaction of 2-methylthio-2-thiazoline with 3,3′-dithiobispropylamine and from 2-methylthio-5,6-dihydro-4H-1,3-thiazine with 2,2′-dithiobisethylamine. In rearrangements that may involve such an intermediate as **4c**, the product containing the five-membered ring is preferentially obtained.

The rearrangement of 2-(2-aminoethylthio)-2-thiazoline to 2-(2-mercaptoethylamino)-2-thiazoline through a postulated bicyclic intermediate, analogous to that proposed by Doherty, *et al.*,[1] for the conversion of 2-(2-aminoethyl)-2-thiopseudourea (AET) to 2-mercaptoethylguanidine, has been reported.[2] Similar reactions involving derivatives of 2-oxazolines, in which rearrangement can proceed through a bicyclic intermediate, have been described.[3] In the present paper the application of rearrangements of this kind to six-membered heterocyclic systems and to compounds with longer side chains is considered.

2-(3-Aminopropylthio)-5,6-dihydro-4H-1,3-thiazine dihydrobromide (**3b**) was prepared by the aminopropylation of tetrahydro-2H-1,3-thiazine-2-thione (**1b**) with 3-bromopropylamine hydrobromide. Treatment of the amine dihydrobromide with sodium hydroxide afforded two crystalline products, one of which precipitated quite rapidly. The properties of the second product, which separated slowly from the reaction mixture, indicated that it was the disulfide (**6b**) of 2-(3-mercaptopropylamino)-5,6-dihydro-4H-1,3-thiazine (**5b**), the mercaptan that would be obtained by rearrangement of **3b** through the bicyclic intermediate **4b**. The identity of the disulfide was established by its synthesis from 2-methylthio-5,6-dihydro-4H-1,3-thiazine (**7b**) and 3,3′-dithiobispropylamine dihydrochloride (**8b**) (see Scheme I).

The product that precipitated rapidly on treatment of **3b** with sodium hydroxide was found to be a base, which formed a dipicrate and which remained unchanged on further exposure to sodium hydroxide. Its infrared spectrum showed strong bands at 6.12 and 6.28 μ comparable to strong absorptions in both 2-methylamino-5,6-dihydro-4H-1,3-thiazine (6.10 μ, N=CN)

SCHEME I



and in 2-methylthio-5,6-dihydro-4H-1,3-thiazine (6.26 μ, N=CS).[4] The nmr spectrum indicated that the molecule contained six methylene groups adjacent to nitrogen or sulfur (set of signals from τ 6.1 to 7.1) and three methylene groups enclosed by methylenes (separate set of signals from τ 7.8 to 8.4). These observations and the analytical data demonstrated that

(1) D. G. Doherty, R. Shapira, and W. T. Burnett, Jr., *J. Am. Chem. Soc.*, **79**, 5667 (1957).

(2) R. C. Clapp, L. Long, Jr., and T. Hasselstrom, *J. Org. Chem.*, **26**, 1666 (1961).

(3) (a) R. C. Clapp, L. Long, Jr., and T. Hasselstrom, *ibid.*, **28**, 1308 (1963); (b) *ibid.*, **29**, 2172 (1964).

(4) Similarly, in 2-thiazolines and 2-oxazolines[2,3] the C=N absorption is characteristically at higher wavelength in 2-alkylthio than in 2-alkylamino derivatives.