Purine Nucleosides. XVI. Synthesis of the Naturally Occurring 2′-O-Methylpurine Ribonucleosides and Related Derivatives[1]

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Abstract: The synthesis of 2′-O-methyladenosine (VII) has been accomplished by methylation of 6-chloro-9-β-D-ribofuranosylpurine (V) with diazomethane followed by treatment of the product with methanolic ammonia. 2′-O-Methylguanosine (IV) has been prepared in several steps *via* the action of diazomethane on 2-amino-6-chloro-9-β-D-ribofuranosylpurine (I) which gave 2′-O-methyl-2-amino-6-chloro-9-β-D-ribofuranosylpurine (II). II was converted to 2′-O-methyl-2-amino-9-β-D-ribofuranosyl-6-purinethione (III) which in turn in the presence of hydrogen peroxide gave 2′-O-methylguanosine. The synthetic 2′-O-methylguanosine proved to be identical with the natural product isolated from yeast soluble ribonucleic acid (s-RNA).

Since Smith and Dunn[2] first isolated 2′-O-methyladenosine from the RNA of wheat germ and rat liver in 1959 the presence of 2′-O-methyl ribonucleosides has been verified as occurring in ribonucleic acid from a wide variety of sources.[3-8] Morisawa and Chargaff[9] examined the alkali-stable dinucleotide fractions of rat liver RNA and yeast-soluble RNA and detected 2′-O-methylguanosine on enzymatic digestion of these fractions. Lane[10] has recently shown that as much as 1.7% of the constituent nucleoside residues in RNA from wheat germ consists of 2′-O-methyl ribonucleosides. Hydrolysis of wheat germ s-RNA reveals that about 1.3% of the nucleosides bear a 2′-O-methyl substituent.[11] Although the biogenesis of O-methylation is presently unknown it should be pointed out that a considerable degree of nonrandomness exists in the amount of O-methylation and in the distribution of the O-methyl ribonucleosides in the ribonucleate chains.[11] It has been noted that the 2′-O-methyl ribonucleoside 5′-phosphates are resistant to bull semen and snake venom 5′-nucleotidases and a number of phosphorylase enzymes.[11,12] This modification in substrate specificity toward RNA hydrolytic enzymes may be of considerable importance in biochemical function.[13a] The fact that the 2′-O-methyl ribonucleosides appear to be widely distributed in nature both in the high and low molecular weight fractions of RNA suggests that these derivatives play an important role in protein biosynthesis. Analysis of the nucleotide sequence in yeast tyrosine transfer ribonucleic acid (t-RNA) has recently revealed 2′-O-methylguanosine as one of the components.[13b] 2′-O-Methylguanosine has also been noted in a serine-specific t-RNA whose nucleotide sequence has recently been determined.[13c] The obvious desirability of obtaining these 2′-O-methyl ribonucleosides in sufficient quantity for possible incorporation prompted the present synthetic investigation. In addition any total chemical synthesis of specific t-RNA molecules would require a synthetic route to the 2′-O-methylpurine ribonucleosides.

Recently 2′-O-methyluridine has been prepared by a direct methylation of 3′,5′-di-O-trityluridine with methyl iodide in the presence of silver oxide.[14] Although extensive methylation of the pyrimidine ring occurred, 2′-O-methyluridine and 2′-O-methylcytidine were successfully separated from other products.[14] In our own laboratory diazomethane has been chosen as a methylating agent and the direct synthesis of 2′-O-methyladenosine from adenosine using this agent has been described in a preliminary report.[15] In the present work the use of diazomethane to achieve selective methylation has been further investigated. Since the chemical synthesis of 2′-O-methylguanosine was one of the major goals several possible routes were investigated. The direct treatment of guanosine in aqueous solution with ethereal diazomethane has been shown to result in the preparation of 7-methylguanosine[16] as the major product. 7-Methylguanosine has similarly been prepared by direct methylation of guanosine with methyl iodide.[17] The detection of 1-methyl-2′-deoxyguanosine as well as 7-methyl-2′-deoxyguanosine has recently been reported[18] by the action of diazomethane on 2′-deoxyguanosine. In the first preliminary experiments 2-amino-6-benzyloxy-9-β-D-ribofuranosylpurine[19] was selected for study. Methylation of 2-amino-6-benzyl-

(1) Supported by Research Grants CA-08109 and CA-08109 from the National Cancer Institute of the National Institutes of Health, Public Health Service.

(2) J. D. Smith and D. B. Dunn, *Biochim. Biophys. Acta.*, **31**, 573 (1959).

(3) R. H. Hall, *Biochemistry*, **3**, 876 (1964).

(4) B. B. Biswas and J. Myers, *Nature*, **186**, 238 (1960).

(5) E. H. Hall, *Biochim. Biophys. Acta*, **68**, 278 (1963).

(6) R. H. Hall, *Biochem. Biophys. Res. Commun.*, **12**, 429 (1963).

(7) H. Singh and B. G. Lane, *Can. J. Biochem.*, **42**, 87 (1964).

(8) K. Ogata, Y. Nakao, S. Igarasi, E. Omura, Y. Sugino, M. Yoneda, and I. Suhara, *Agr. Biol. Chem.* (Tokyo), **27**, 110 (1963).

(9) S. Morisawa and E. Chargaff, *Biochim. Biophys. Acta*, **68**, 147 (1963).

(10) B. G. Lane, *Biochemistry*, 4, 212 (1965).

(11) L. Hudson, M. Gray and B. G. Lane, *ibid.*, 4, 2009 (1965).

(12) M. Honjo, Y. Kanai, Y. Furukawa, Y. Mizuno, and Y. Sanno, *Biochim. Biophys. Acta*, **87**, 698 (1964).

(13) (a) R. H. Hall, *Biochemistry*, 4, 661 (1965); (b) J. T. Madison, G. A. Everett, and H. King, *Federation Proc.*, **25**, 519 (1966); (c) H. G. Zachau, D. Dütting, and H. Feldman, *Angew. Chem.*, **78**, 392 (1966).

(14) Y. Furukawa, K. Kobayashi, Y. Kanai, and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **13**, 1273 (1965).

(15) A. D. Broom and R. K. Robins, *J. Am. Chem. Soc.*, **87**, 1145 (1965).

(16) J. A. Haines, C. B. Reese, and Lord Todd, *J. Chem. Soc.*, 5281 (1962).

(17) J. W. Jones and R. K. Robins, *J. Am. Chem. Soc.*, **85**, 193 (1963).

(18) O. M. F. Friedman, G. N. Mahapatra, B. Dash, and R. Stevenson, *Biochim. Biophys. Acta*, **103**, 286 (1965).

(19) J. F. Gerster and R. K. Robins, *J. Am. Chem. Soc.*, **87**, 3752 (1965).

oxy-9-β-D-ribofuranosylpurine with diazomethane in aqueous dimethoxyethane resulted in two major products with similar ultraviolet absorption spectra, $\lambda_{max}^{pH\ 1}$ 270 mμ and $\lambda_{max}^{pH\ 11}$ 264.5 mμ. These compounds proved to be ring-opened 5-methylformamidopyrimidine derivatives which are similar to those isolated from ring-opened 7-methylguanosine.[20] Thus although the presence of 2′-O-methylribose could be detected in the acid hydrolysates of the crude reaction mixture, methylation had also occurred on position 7. An attempt was then made to select a derivative of guanosine that would *not* undergo methylation in the purine ring under the reaction conditions employed. 2-Amino-6-chloro-9-β-D-ribofuranosylpurine[21,22] (I) was then treated with diazomethane in aqueous dimethoxyethane. The crude product obtained exhibited no change in the ultraviolet absorption spectrum which was strongly indicative of methylation in the sugar moiety *only*. It should be noted that 2-amino-6-chloro-9-β-D-ribofuranosylpurine (I) *does* methylate on N_7 in the presence of methyl iodide in dimethylformamide.[23] 2′-O-Methyl-2-amino-6-chloro-9-β-D-ribofuranosylpurine (II) was obtained as a glass which could not be readily purified by crystallization. The reaction product was therefore converted directly to 2′-O-methyl-2-amino-9-β-D-ribofuranosyl-6-purinethione (III) by the use of methanolic sodium hydrosulfide.[24] Crude III was recrystallized from hot water and finally purified by alumina chromatography. The main contaminant provided to be 2-amino-9-β-D-ribofuranosyl-6-purinethione which remained adsorbed on the column when eluted with an ethyl alcohol–*n*-propyl alcohol–water system (4:1:2, v/v). This procedure gave pure 2′-O-methyl-2-amino-9-β-D-ribofuranosyl-6-purinethione (III). Hydrogen peroxide and III in the presence of aqueous ammonia[25a] gave 2′-O-methylguanosine (IV) in good yield. The presence of the 2′-O-methyl group was indicated by a three-proton sharp singlet at δ 3.4 (d_6-DMSO). Acid hydrolysis gave 2′-O-methylribose which was confirmed by paper chromatography. 2′-O-Methylguanosine exhibited the same $R_{guanosine}$ values in three systems as those described by Hall[5] for the natural product.[25b] Since the presence of the 6-chloro group had prevented methylation of the heterocyclic ring nitrogens it became of interest to study the methylation of 6-chloro-9-β-D-ribofuranosylpurine[21,22,26] (V) itself with diazomethane. Similar treatment of V with diazomethane in aqueous 1,2-dimethoxyethane gave crude 2′-O-methyl-6-chloro-9-β-D-ribofuranosylpurine (VI) which was treated directly with methanolic ammonia to give crude 2′-O-methyladenosine (VII). The main contaminant was adenosine which was separated by the use of a basic ion-exchange column (Dowex-1) according to the procedure of Dekker.[27] The 2′-O-methyladenosine prepared by this procedure was found to be identical with the product previously obtained by direct methylation of adenosine with diazomethane.[15] It should be noted that the direct methylation of adenosine to give VII is the preferred synthetic procedure. Attempts to purify and crystallize 2′-O-methyl-6-chloro-9-β-D-ribofuranosylpurine (VI) were not rewarding. The high reactivity of the chloro group toward nucleophilic substitution prevented the application of several separation and purification procedures. The crude glass (VI) which consisted of some unreacted 6-chloro-9-β-D-ribofuranosylpurine and the 2′-O-methyl derivative was treated directly with alcoholic sodium hydrosulfide to yield crude 2′-O-methyl-9-β-D-ribofuranosyl-6-purinethione (VIII). The desired product (VIII) was readily purified by alumina chromatography to give 2′-O-methyl-9-β-D-ribofuranosyl-6-purinethione in over 70% yield from 6-chloro-9-β-D-ribofuranosylpurine (V). The identity of VIII was further confirmed by treatment with hydrogen peroxide and aqueous ammonia to give 2′-O-methylinosine (IX) which proved to be identical with 2′-O-methylinosine previously prepared from 2′-O-methyladenosine.[15]

The amazing selectivity of O-methylation in the present study is further demonstrated by the fact that 2′-deoxyadenosine under the same reaction conditions as adenosine[15] did not yield any O-methylated products. The starting 2′-deoxyadenosine was recovered quantitatively unchanged. Izatt, *et al.*,[28] have proposed that two adjacent *cis* hydroxyl groups are a necessary structural feature for the acidic character of adenosine (pK = 12.35). Although the presence of the corresponding 3′-O-methylpurine ribonucleosides has not been entirely eliminated in the present work, this isomer if present in the crude reaction mixture is in very small quantity relative to the 2′-O-methyl derivative. The 2′-O-methylpurine nucleosides prepared in the present work were shown to give only one spot in at least three solvent systems.

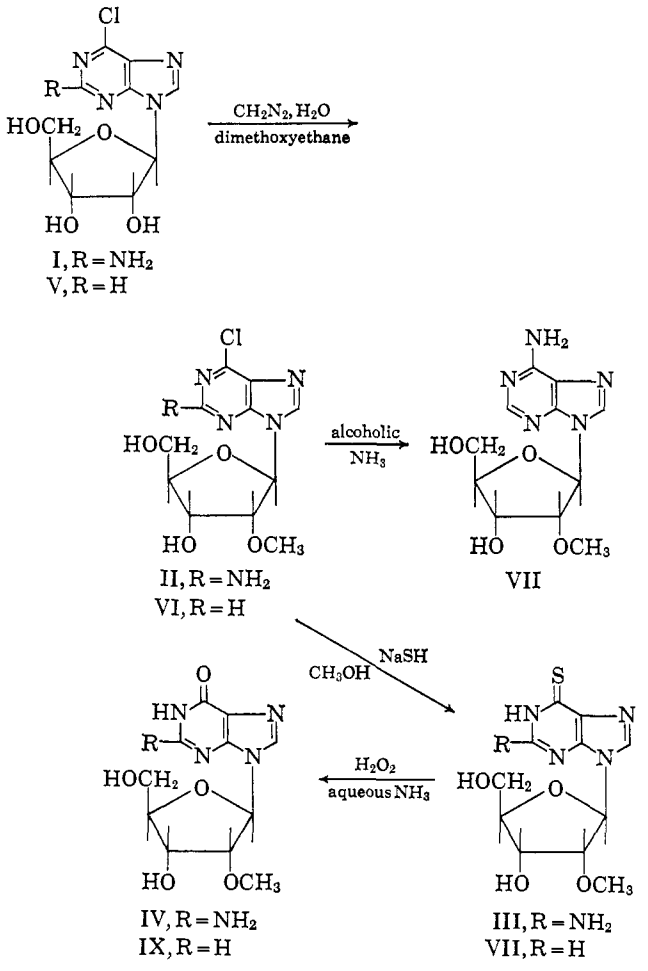
It has recently been shown[14] that methylation at the 2′ position of the pyrimidine ribonucleosides increases the stability of the glycosyl linkage to acid hydrolysis. Many cell systems possess degradative enzymes which rapidly cleave purine nucleosides,[29–31] to the purine base and ribose 1-phosphate. It will be interesting to see if some of the presently prepared 2′-O-methylpurine ribonucleosides are resistant to and in fact do inhibit this type of enzymatic phosphorolysis similar to that noted in the case of the corresponding nucleotides[11,12] (see Scheme I).

Experimental Section

Preparation of Diazomethane in 1,2-Dimethoxyethane. A mixture of 40% aqueous sodium hydroxide (250 ml) and 1,2-dimethoxyethane (500 ml) was cooled to −15°. To this stirred (ice cold) solution was carefully added over a 15-min period 100 g of N-nitrosomethylurea. The solution was stirred for 1 hr and the yellow dimethoxyethane solution of diazomethane was carefully

(20) L. B. Townsend and R. K. Robins, *J. Am. Chem. Soc.*, **85**, 242 (1963).
(21) R. K. Robins, *ibid.*, **82**, 2654 (1960).
(22) J. F. Gerster, J. W. Jones, and R. K. Robins, *J. Org. Chem.*, **28**, 945 (1963).
(23) A. D. Broom and R. K. Robins, *J. Heterocyclic Chem.*, 1, 113 (1964).
(24) J. A. Johnson, Jr., H. J. Thomas, and H. J. Schaeffer, *J. Am. Chem. Soc.*, **80**, 699 (1958).
(25) (a) J. F. Gerster and R. K. Robins, *J. Org. Chem.*, in press. (b) The authors wish to thank Dr. Ross H. Hall for a further independent comparison in his laboratory, of our synthetic 2′-O-methylguanosine with the natural material. Dr. Hall found the compounds to possess identical R_f values in five different solvent systems.
(26) R. K. Robins, *Biochem. Prepn.*, 10, 145 (1963).
(27) C. A. Dekker, *J. Am. Chem. Soc.*, **87**, 4027 (1965).
(28) R. M. Izatt, L. D. Hansen, J. H. Rytting, and J. J. Christensen, *ibid.*, **87**, 2760 (1965).
(29) A. M. Gotto, A. W. Meikle, and O. Touster, *Biochim. Biophys. Acta*, **80**, 552 (1964).
(30) A. R. P. Paterson and A. Sutherland, *Can. J. Biochem.*, **42**, 1415 (1964).
(31) G. A. Lepage and I. G. Junga, *Cancer Res.*, **23**, 739 (1963).

Scheme I



decanted and allowed to stand over solid sodium hydroxide pellets at −15° overnight. The diazomethane solution was carefully filtered to remove all particles of solid sodium hydroxide. The solution of diazomethane, approximately 600 mmoles, was then dry, neutral, and ready for use.[32]

Preparation of 2′-O-Methyladenosine (VII) from 6-Chloro-9-β-D-ribofuranosylpurine (V). One gram of 6-chloro-9-β-D-ribofuranosylpurine[26] (VI) was dissolved in hot water (100 ml), and the stirred solution was treated with diazomethane (approximately 120 mmoles) in 1,2-dimethoxypropane (125 ml) (the temperature of the reaction media gradually dropped from 70 to 40°). After 2 hr, the yellow color of the reaction mixture was discharged, and the solution was evaporated to dryness under reduced pressure (bath temperature 40°). The residual gum was twice dissolved in absolute ethanol and evaporated under reduced pressure. The residue was then dissolved in cold, saturated methanolic ammonia (80 ml) and placed in a sealed pressure bottle. The solution was heated at 90° for 10 hr and the solvent evaporated. The residue was examined by paper chromatography and it was shown to exhibit two major ultraviolet absorbing spots corresponding to adenosine and 2′-O-methyladenosine. The gum was then dissolved in water (5 ml) and adsorbed on Dowex 1-X2 (OH^-) (200–400 mesh) column (2.5 × 27 cm). The column was eluted with distilled water and 50-ml fractions were collected. Fractions 5–7 gave a compound (about 4 mg) with an ultraviolet absorption spectrum similar to adenosine, but possessing a higher R_f than 2′-O-methyladenosine. Fractions 17–24 gave the desired 2′-O-methyladenosine which was purified by recrystallization from absolute ethyl alcohol. The product was further identified by paper chromatography and rigorous comparison with 2′-O-methyladenosine prepared[15] by methylation of adenosine. The product (210 mg) melted at 201–202°. Acid hydrolysis revealed the presence of 2′-O-methyl-D-ribose (confirmed by paper chromatography). The pmr spectrum was identical with the authentic sample[15] (3 H singlet at δ 3.78 due to 2′-O-methyl group, $[\alpha]^{23}D$ −57.9° (*c* 1, H_2O)).

2′-O-Methyl-9-β-D-ribofuranosyl-6-purinethione (VIII). Five grams of 6-chloro-9-β-D-ribofuranosylpurine[26] (VI) was dissolved in hot, distilled water (200 ml). To the stirred solution a solution of diazomethane (600 mmoles approximately) in 650 ml of 1,2-dimethoxyethane was added in three major portions over a 30-min period. After 4 hr the colorless reaction solution was evaporated to dryness on a rotary evaporator at room temperature. The residue was dissolved in 30 ml of absoute ethyl alcohol and evaporated to dryness. The process was repeated with 30 ml of methyl alcohol. The residue was finally dissolved in 100 ml of absolute methyl alcohol. To this solution was added 35 ml of methanolic hydrogen sulfide[24] and the solution was gently refluxed for 15 min. The resulting solution was evaporated to dryness and the residue was dissolved in 20 ml of hot water and neutralized (pH 5) with acetic acid. The yellow precipitate was cooled overnight and filtered. The crude product was dissolved in a minimum of water and adsorbed on alumina (100 g of Woelm, neutral, column 2.5 cm in diameter). The column was eluted with the solvent system consisting of ethyl alcohol–*n*-propyl alcohol–water (4.1:2, v/v). Thirty-milliliter fractions were collected. Fractions 3–19 were evaporated to give 4.79 g of product which was rechromatographed on a second similar alumina column. The first 60 ml of the eluent contained a small amount of a faster moving spot, R_f 0.89 compared to R_f 0.52 for VIII and R_f 0.06 for 9-β-D-ribofuranosyl-6-purinethione (tlc using 0.25-mm alumina-H and ethyl alcohol–*n*-propyl alcohol–water, 4:1:2, v/v). Fractions 3–13 gave pure 2′-O-methyl-9-β-D-ribofuranosyl-6-purinethione (VIII). This product was dissolved in methyl alcohol and precipitated with absolute ether to give 3.58 (71.24%) of analytically pure solid, uncontaminated with 9-β-D-ribofuranosyl-6-purinethione.

Anal. Calcd for $C_{11}H_{14}N_4O_4S$: C, 44.3; H, 4.7; N, 18.8. Found: C, 44.1; H, 4.8; N, 18.6.

VIII gradually decomposes above 200° and can be successfully crystallized from absolute methanol. Acid hydrolysis gave 2′-O-methyl-D-ribose as the only detectable sugar. Ultraviolet absorption showed $\lambda_{max}^{pH\,1}$ 320 mμ (ε 24,150) and 225 mμ (ε 9095), $\lambda_{max}^{pH\,11}$ 309.5 mμ (ε 23,260) and 233 mμ (ε 15,210), $[\alpha]^{22}D$ −53.5° (*c* 0.42, 0.1 *N* sodium hydroxide). Proton magnetic resonance spectra (d_6-DMSO) showed a 3 H singlet at δ 3.44 (2′-O-methyl group).

2′-O-Methyl-2-amino-9-β-D-ribofuranosylpurine-6-thione (III). 2-Amino-6-chloro-9-β-D-ribofuranosylpurine[22] (I, 5.97 g) was dissolved in 165 ml of hot, distilled water. To the warm stirred solution was carefully added 900 ml of 1,2-dimethoxyethane containing 720 mmoles of diazomethane prepared from 120 g of N-nitrosomethylurea. The stirred solution was allowed to cool to room temperature and after 4 hr the solution was evaporated to dryness at 40° under reduced pressure. To the residue was added 35 ml of absolute ethyl alcohol and the solution was evaporated under reduced pressure. The process was repeated with 200 ml of absolute ether. The pale glass (6.5 g) was suspended in dry methyl alcohol (100 ml) which had been previously saturated with hydrogen sulfide. The mixture was then treated with 43.5 ml of 1 *N* methanolic sodium hydrogen sulfide[24] heated in a pressure bottle at 85°. After 1 hr the reaction solution was cooled to room temperature and evaporated to dryness on a rotary evaporator. The residue was dissolved in 40 ml of hot, distilled water and filtered. The pale filtrate was neutralized (pH of 6) with acetic acid. The solution was cooled overnight and filtered. The crude product was recrystallized from 20 ml of hot water. The yellow, crystalline product was then dissolved in hot water and adsorbed on an alumina column (Woelm, neutral, 100 g, 2.5 cm). The column was eluted with the same solvent system as for VIII and 25-ml fractions were collected. Fractions 3 and 4 contained the desired product III and a small amount of product which had a high R_f, 0.85, as compared to R_f 0.68 for III and R_f 0.07 for 2-amino-9-β-D-ribofuranosyl-6-purinethione (tlc using 0.25-mm alumina-H and ethyl alcohol–*n*-propyl alcohol–water, 4:1:2, v/v). Fractions 5–40 gave pure 2′-O-methyl-2-amino-9-β-D-ribofuranosylpurine-6-thione (III, 3.58 g), 57.1% uncontaminated by 2-amino-9-β-D-ribofuranosylpurine-6-thione. The product was recrystallized from water to yield silver crystals, mp 234°. The product was heated at 100° under vacuum for 4 hr for analysis.

Anal. Calcd for $C_{11}H_{15}N_5O_4$: C, 42.2; 4.8; N, 22.4. Found: C, 42.0; H, 4.9; N, 22.1.

Ultraviolet absorption showed $\lambda_{max}^{pH\,1}$ 342 mμ (ε 13,780); $[\alpha]^{26}D$

(32) The presence of excess sodium hydroxide is especially deleterious when methylating the 6-chloropurine nucleosides.

$-51.8°$ (c 0.50, 0.1 N sodium hydroxide). The pmr spectrum (d_6-DMSO) exhibited a 3 H singlet at δ 3.48 (2′-O-methyl group). Acid hydrolysis gave the same sugar as 2′-O-methyladenosine.[15]

2′-O-Methylguanosine (IV). 2′-O-Methyl-2-amino-9-β-D-ribofuranosyl-6-purinethione (III, 314 mg) was dissolved in 5 ml of 20% aqueous ammonia. To the cold solution was added 0.8 ml of 30% hydrogen peroxide. The solution was left at room temperature for 4 hr and then evaporated to dryness on a rotary evaporator. The remaining white powder was dissolved in 5 ml of water and placed on an alumina column (40 g, Woelm, neutral, 2.5 cm). The column was eluted with the same solvent system as employed in the purification of VII and 25-ml fractions were collected. Fractions 2–5 were combined and evaporated to dryness. The residue was recrystallized from absolute methyl alcohol to give 150 mg of pure 2′-O-methylguanosine (IX), mp 218–220°.

Anal. Calcd for $C_{10}H_{13}N_5O_5$: C, 44.4; H, 5.1; N, 23.5. Found: C, 44.2; H, 5.2; N, 23.3.

Ultraviolet absorption showed $\lambda_{max}^{pH\ 1}$ 255.5 mμ (ε 10,660), $\lambda_{max}^{pH\ 11}$ 258 mμ (ε 9776), $[\alpha]^{22}D$ $-38.4°$ (c 0.595, water). The pmr spectrum (d_6-DMSO) exhibited a three-proton singlet at δ 3.40 (2′-O-methyl group). Acid hydrolysis showed 2-O-methyl-D-ribose as the only sugar present. The $R_{guanosine}$ values for 2′-O-methylguanosine were identical with those recorded by Hall[5] in the systems A, C, and D for the naturally occurring product.

Chemistry and Metabolism of Sphingolipids. 3-Oxo Derivatives of N-Acetylsphingosine and N-Acetyldihydrosphingosine

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Abstract: N-Acetylsphingosine and N-acetyldihydrosphingosine were prepared in high yields by reaction of the free bases with acetic anhydride in methanol. Selective oxidations of the secondary hydroxyl groups in these N-acetyl derivatives with chromic anhydride in benzene–pyridine yielded the previously undescribed 3-oxo compounds. Reductions of the 3-oxo compounds with sodium borodeuteride yielded monodeuterated *erythro* and *threo* forms of the N-acetyl bases; mass spectra confirmed the location of deuterium on C_3. Some N-acetyldihydrosphingosine-3,5-d_3 was formed by 1,4 addition in the reduction of 3-oxo-1-hydroxy-2-acetamido-4-octadecene.

Sphingosine (I) is representative of a group of related long-chain aliphatic 2-amino-1,3-diols which are basic structural constituents of all sphingolipids found in animal tissues. The predominant base may be sphingosine or the C_{20} homolog, occurring in mixtures with smaller quantities of their saturated forms, dihydrosphingosine and C_{20}-dihydrosphingosine. Sphingolipid bases have the D-*erythro* configuration; the double bond in sphingosine is *trans*.[1] Several methods have been reported for the chemical synthesis of long-chain bases of this type.[2,3]

The biosynthesis of sphingolipid bases in animal tissues is believed to involve condensation of hexadecanal with serine–pyridoxal phosphate and simultaneous or subsequent decarboxylation, yielding dihydrosphingosine.[4] Subsequent dehydrogenation yields sphingosine.[4] Recent studies by Greene, Kaneshiro, and Law[5] have shown that the synthesis of phytosphingosine and other sphingolipid bases in whole yeast cells is similar to the pathway for sphingosine and dihydrosphingosine in animal systems.

(1) Studies on the stereochemistry of sphinogosine and related bases were reported by several investigators. See H. E. Carter, D. S. Galanos, and Y. Fujino, *Can. J. Biochem. Physiol.*, 34, 320 (1956); M. Prostenik, M. Munk-Weinert, and D. E. Sunko, *J. Org. Chem.*, 21, 406 (1956).

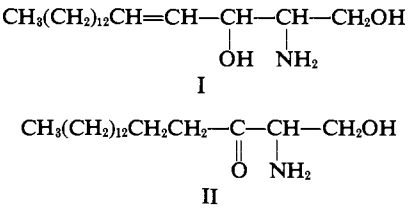
(2) See, for example, D. Shapiro and T. Sheradsky, *ibid.*, 28, 2157 (1963).

(3) See, for example, D. Shapiro, H. Segal, and H. M. Flowers, *J. Am. Chem. Soc.*, 80, 2170 (1958), and references cited therein.

(4) R. O. Brady, J. V. Formica, and G. J. Koval, *J. Biol. Chem.*, 233, 1072 (1958).

(5) M. L. Greene, T. Kaneshiro, and J. H. Law, *Biochim. Biophys. Acta*, 98, 582 (1965).

The possibility has not been excluded that a 3-oxo compound (II) may be an intermediate in the biosynthetic pathway as proposed by Sprinson and Coulon some years ago.[6] Based on experiments with serine-H,[3]



I

II

Weiss was led to speculate that 3-oxo forms of the bases may be intermediates.[7] Although chemically related to the sphingolipid bases, the ketones are not presently known to be of biological origin. Their possible existence in sphingolipids remains obscure, however, since they do not survive the hydrolytic conditions commonly used to liberate long-chain bases from their parent sphingolipids.

Since the chemical and chromatographic properties of 3-oxo compounds related to sphingosine and dihydrosphingosine had not been described previously, methods for their chemical synthesis have been investigated. The present report describes the synthesis of the stable N-acetyl derivatives of 3-oxo-1-hydroxy-2-aminooctadecane and 3-oxo-1-hydroxy-2-amino-4-octadecene by selective oxidation of secondary OH groups with

(6) D. B. Sprinson and A. Coulon, *J. Biol. Chem.*, 207, 585 (1954).

(7) B. Weiss, *ibid.*, 238, 1953 (1963).