Carbohydrate Research, 127 (1984) 141–145
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

The preparation of a crystalline guanosine trialcohol and its sodium salt

LEON M. LERNER

Department of Biochemistry, State University of New York, Downstate Medical Center, Brooklyn, New York 11203 (U.S.A.)

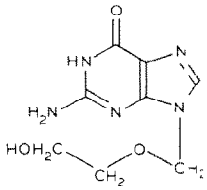
(Received February 23rd, 1983; accepted for publication June 17th, 1983)

The preparation of several nucleoside trialcohols has been reported from this laboratory[1]. Although these compounds were known, either as impure solutions or lyophilized products[2–5], this was the first time that the trialcohols derived from adenosine, cytidine, and uridine, and their derivatives had been obtained in pure form by use of established preparative techniques of organic chemistry. Later on, other nucleoside trialcohols and dialcohols were prepared, many in crystalline form, in order to study their binding properties to the enzyme, adenosine deaminase[6]. Noticeably lacking[1,6] was the preparation of guanosine trialcohol, 1-(1,3-dihydroxy-2-propyl)-1-(guanin-9-yl)-1(*R*),2-ethanediol (**2**). At the time, the problem dealt mainly with the peculiar physical properties of guanosine and the dialdehyde formed upon oxidation with sodium periodate, which caused many technical difficulties. Indirect methods had been used to obtain small amounts of guanosine trialcohol for biochemical experiments[2–4], but the preparation of significant quantities of this substance by a straightforward route has been elusive. Recently, 9-(2-hydroxyethoxymethyl)guanine, commonly called acyclovir (**1**, trade name Zovirax), has been found to be a potent antiherpes drug of low toxicity[7], and is now being used clinically to treat genital herpes infections. Since the work described in this report was begun, a related compound, 9-{[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl}guanine has been reported to be a good antiviral agent[8]. The close structural similarity of guanosine trialcohol (**2**) to acyclovir (**1**) prompted a reinvestigation of its preparation and the development of the procedure described herein.

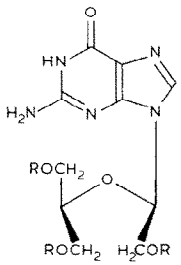
The key to the first step of the synthesis of guanosine trialcohol (**2**) was the use of periodic acid for the oxidative cleavage of the glycol[2,9], rather than sodium periodate. In the earlier attempts, iodate was removed with an anion-exchange column in the acetate form[2,9], followed by reduction of the aldehyde groups with sodium borohydride. However, attempts to obtain a pure, crystalline material

0008-6215/84/$ 03.00

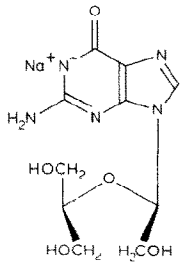
failed, and heavy losses were incurred during subsequent attempts at chromatography. The successful route described herein involves acetylation of the alcohol groups, removal of salts by partitioning between an organic solvent and water, and crystallization of the triacetate derivative **3**. The removal of a significant proportion of inorganic salts prior to reduction was achieved by ethanol precipitation rather



Acyclovir (1)



2 R = H
3 R = Ac



4

than a laborious and lengthy chromatography (Method A). However, it was not necessary to remove any inorganic salts in order to perform the acetylation (Method B). In fact, for an unknown reason, the product obtained in Method B was much purer than that described in Method A, where colored by-products had to be removed with activated charcoal. An attempt to prepare the tribenzoate with benzoyl chloride in pyridine yielded an intractable black tar. It is of interest that removal of the acetyl groups with sodium methoxide solution yielded crystals in the form of the sodium salt (**4**) of guanosine trialcohol. Compound **4** was also obtained by recrystallization of **2** from methanolic sodium methoxide solution.

EXPERIMENTAL

General methods. — Melting points were determined with a Kofler hot-stage and correspond to corrected values. ^{1}H-N.m.r. spectra were recorded for solutions in di($^2H_3$)methyl sulfoxide with a Varian T-60A spectrometer and tetramethylsilane as the internal reference, u.v. spectra with a Beckman Model 25 spectrophotometer, and i.r. spectra with a Perkin–Elmer Model 21 spectrophotometer and used primarily to establish the identity of various crops of crystals. Optical rotations were determined for solutions in 100-mm length cells with a Perkin–Elmer Model 141 polarimeter. Evaporations were performed under reduced pressure in a rotary evaporator at the bath temperatures designated. Elemental analyses were performed by the Spang Microanalytical Laboratory, Eagle Harbor, MI 49951. Guanosine, homogeneous by paper chromatography, was purchased from Aldrich Chemical Co., Milwaukee, WI 53233, used directly and without recrystallization.

*2-Acetoxy-1-(1,3-diacetoxy-2-propyl)-1-(guanin-9-yl)-1(*R*)-ethane.* (**3**). — *Method A.* Guanosine (10 g, 35 mmol) was suspended in 0.1M periodic acid (385

mL) and stirred for 3 h at room temperature, protected from light. The solution was poured into stirring ethanol (1 L) containing M aq. sodium hydroxide solution (38.5 mL). Stirring was continued for 1.5 h and the precipitate removed by suction filtration on a pad of filter aid. The pad was washed with ethanol (200 mL) and the filtrate treated with a solution of sodium borohydride (8 g) in water (200 mL). After 17 h of stirring in the dark, the pH was adjusted to ~5 (pH paper) with Amberlite IR-120 (H^+) ion-exchange resin. The resin was removed by filtration on a glass funnel and washed several times with water. The solution was evaporated (40°) to dryness and coevaporated with dry pyridine (3 × 75 mL). The residue was suspended in dry pyridine (150 mL) and acetic anhydride (30 mL) was added. The mixture was stirred vigorously for 64 h. The orange solution was chilled in an ice-bath, ethanol (40 mL) was added dropwise (30 min), and after 30 min at 0°, the mixture was stirred at room temperature for 3 h. The solution was evaporated (40°) and the residue coevaporated with toluene (4 times) to remove pyridine. The residue was dissolved in chloroform (150 mL), the solution washed with water (3 × 200 mL) and dried (anhydrous magnesium sulfate). After filtration and evaporation, the oily residue was subjected to one more evaporation with toluene, whereupon the product solidified. It was dissolved in a mixture of boiling ethanol (~70 mL) and chloroform (~25 mL). The latter was boiled off and crystallization was allowed to proceed at room temperature. Filtration by suction afforded 5.57 g (38%) of tan-colored product. A second crop (0.136 g, 1%) was obtained by concentration of the mother liquor. The first crop was recrystallized from ethanol–chloroform as just described, except that activated charcoal (Darco G-60 removed the color whereas Norit A did not) was used because the crystals otherwise absorbed the colored impurities. The yield was 5.20 g of white crystals, m.p. 193–194°. The m.p. was raised to 194.5–195.5° by one more recrystallization, and the product was identical (m.p., i.r., and n.m.r.) to that described under Method B.

Method B. Guanosine (10 g, 35 mmol) was treated with periodic acid as in Method A. Sodium hydroxide (38.5 mL of M solution) was added. Before the solution completely gelled, sodium borohydride (8 g) dissolved in water (200 mL) was added, and the mixture maintained near room temperature with the aid of an ice–water bath. The solution was then stirred for 18 h at room temperature, protected from light. The pH was adjusted to ~6 with glacial acetic acid, and the solution evaporated (40°) to a syrupy residue. The residue was coevaporated with dry pyridine (3 ×), and then suspended in dry pyridine (150 mL) and treated with acetic anhydride (40 mL). The mixture was stirred vigorously for 40 h at room temperature, and then filtered through a plug of glass wool to remove insoluble inorganic material. The filtrate was chilled in an ice-bath and treated with ethanol (60 mL), added dropwise over 2 h. The mixture was then stirred overnight at room temperature. The solvents were evaporated (40°), and the excess pyridine was evaporated with the aid of toluene (3 ×). The residue was dissolved in chloroform (150 mL), and the solution washed with 50% aqueous sodium chloride solution (3 × 100 mL) and dried (anhydrous magnesium sulfate). Filtration, evaporation of the solvent,

and coevaporation with toluene gave a solid residue that was crystallized as described in Method A, to yield 5.07 g (35%) of white crystals, m.p. 193–194°. One recrystallization afforded 4.63 g of **3** as large, white prismatic crystals; m.p. 194.5–195.5°; $[\alpha]_D^{25}$ +35.8° (*c* 1.04, *N,N*-dimethylformamide); ^{1}H-n.m.r.: δ 10.68 (br. s, 1 H, NH), 7.92 (s, 1 H, H-8), 6.52 (br. s, 2 H, NH_2), 5.87 (t, 1 H, NCH), 4.53, 4.20, 3.93 (ms, 7 H, CH_2 and OCH), 2.08, 1.97, and 1.77 (3 s, 9 H, 3 CH_3CO).

Anal. Calc. for $C_{16}H_{21}N_5O_8$: C, 46.71; H, 5.15; N, 17.03. Found: C, 46.87; H, 5.11; N, 16.97.

*1-(1,3-Dihydroxy-2-propyl)-1-(guanin-9-yl)-1(*R*),2-ethanediol (guanosine trialcohol)* (**2**). — The triacetate **3** (1 g) was placed in a pressure bottle and a solution of ammonia in methanol (50 mL), which had been saturated at 0°, was added. The compound dissolved in a few min, and the bottle was sealed and gently shaken for 20 h at room temperature. Some crystals had deposited on the walls and the bottle was chilled in an ice-bath for 4 h. The crystals were filtered off and washed with ice-cold methanol (428 mg). The mother liquor was evaporated (40°) to dryness and the residue dissolved in boiling ethanol (~80 mL). The solution was concentrated to ~20 mL by boiling, and kept for two days at room temperature while crystallization slowly took place. An additional crop (216 mg) was obtained for a total yield of 644 mg (93%). Each crop of crystals was separately recrystallized from ethanol by dissolution in boiling ethanol, followed by concentration of the boiling solution to about one-fourth of its original volume. After being kept for several days, the crystals were scraped off the walls, filtered off, and dried *in vacuo*. Recrystallization of the first crop afforded 363 mg of the analytical sample, melting at 215–220°, and then rehardening (plastic appearance) without remelting (up to 360°), dec. >260°, $[\alpha]_D^{25}$ +43.9° (*c* 1.13, water); $\lambda_{max}^{pH\ 1}$ 256 nm (ε 11 790), $\lambda_{min}^{pH\ 1}$ 227.5 nm (ε 2510), $\lambda_{max}^{H_2O}$ 253 nm (ε 12 900), $\lambda_{min}^{H_2O}$ 221.5 nm (ε 2400), $\lambda_{max}^{pH\ 13}$ 263 nm (ε 10 730), $\lambda_{min}^{pH\ 13}$ 231 nm (ε 4280); ^{1}H-n.m.r.: δ 10.7 (br. s, 1 H, NH), 7.85 (s, 1 H, H-8), 6.50 (br. s, 2 H, NH_2), 5.70 (t, 1 H, NCH), 5.15 (t, 1 H, OH), 4.83–4.67 (overlapping ms, 2 H, 2 OH), 3.83, 3.55, and 3.32 (m, 7 H, 3 CH_2 and OCH).

Anal. Calc. for $C_{10}H_{15}N_5O_5$: C, 42.10; H, 5.30; N, 24.55. Found: C, 42.28; H, 5.32; N, 24.44.

Sodium salt (**4**) *of* **2**. — To a mixture containing **3** (2 g) in methanol (60 mL) was added M methanolic sodium methoxide solution (6 mL). The crystals dissolved after swirling for 5 min, and then the flask was gently shaken for 20 h at room temperature (within 1 h crystals began to appear). The flask was chilled in an ice-bath for 4 h, and the crystals were filtered off, washed with ice-cold methanol, and dried *in vacuo*. The yield of **4** was 1.10 g (74%), m.p. 262.5–264° (dec.) with some decomposition beginning at 250°, $[\alpha]_D^{25}$ +38.0° (*c* 1.24, water); ^{1}H-n.m.r. spectrum similar to that of **1**, except that the peaks for the OH groups were less clearly separated and defined; more importantly, the peak at δ 10.7 had disappeared indicating that the sodium ion was associated with N-1 in place of the hydrogen atom. A flame test with a platinum wire on both the product and an ignition sample was positive for sodium. The residue from the ignition on a platinum foil was alkaline when dis-

solved in water and tested with pH paper, and effervesced vigorously when dilute hydrochloric acid was added.

Anal. Calc. for $C_{10}H_{14}N_5NaO_5$: C, 39.09; H, 4.59; N, 22.80. Found: C, 39.26; H, 4.87; N, 22.96.

In a separate experiment, **2** (100 mg) was suspended in methanol (10 mL) and M methanolic sodium methoxide (1 mL) was added. The crystals did not dissolve until a little heat was applied from a steam bath. The solution was seeded and kept for 2 days at room temperature, and then for 2 days in a refrigerator. The crystals were scraped off the walls of the flask, filtered off, and washed with ice-cold methanol. After being dried *in vacuo,* the crystals weighed 45 mg and were identical in every way to **4** as just described.

REFERENCES

1 L. M. LERNER, *Carbohydr. Res.*, 13 (1970) 465–469.
2 J. X. KHYM AND W. E. COHN, *J. Am. Chem. Soc.*, 82 (1960) 6380–6386.
3 U. L. RAJBHANDARY, *J. Biol. Chem.*, 243 (1968) 556–564.
4 S. H. LEPPLA, B. BJORAKER, AND R. M. BOCK, *Methods Enzymol.*, 12B (1968) 236–240.
5 M. VISCONTINI, D. HOCK, AND P. KARRER, *Helv. Chim. Acta*, 38 (1955) 642–645.
6 L. M. LERNER AND R. R. ROSSI, *Biochemistry*, 11 (1972) 2772–2777; R. R. ROSSI AND L. M. LERNER, *J. Med. Chem.*, 16 (1973) 457–460.
7 H. J. SCHAEFFER, L. BEAUCHAMP, P. DE MIRANDA, G. B. ELION, D. J. BAUER, AND P. COLLINS, *Nature (London)*, 272 (1978) 583–585; D. BRIGDEN, P. FIDDIAN, A. E. ROSLING, AND T. RAVENSCROFT, *Antiviral Res.*, 1 (1981) 203–212.
8 K. O. SMITH, K. S. GALLOWAY, W. L. KENNELL, K. K. OGILVIE, AND B. K. RADATUS, *Antimicrob. Agents Chemother.*, 22 (1982) 55–61.
9 A. J. GRANT AND L. M. LERNER, *Biochemistry*, 18 (1979) 2838–2842.