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Note

Preparation of trialcohols and some of their derivatives from nucleosides

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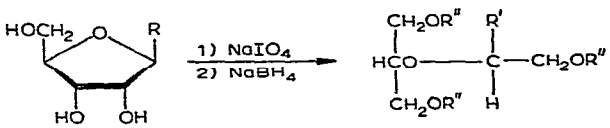
The nucleoside trialcohols have been known for at least a decade, and studies have been made of some of their chemical characteristics[1]. However, they have not been obtained as analytically pure compounds and therefore could not be completely characterized nor their potential biological properties be studied. For example, they may be potential inhibitors of enzymes of the nucleic acid metabolism, or can be used as reference compounds in the periodate oxidation–reduction of the terminal end of RNA[2]. The preparation, purification, and chemical characterization of the nucleoside trialcohols derived from adenosine, uridine, and cytidine, are the subjects of this report.



1 R = uracil-1-yl
2 R = adenin-1-yl
3 R = cytosin-1-yl
4 R′ = uracil-1-yl; R″ = H
5 R′ = uracil-1-yl; R″ = *p*-nitrobenzoyl
6 R′ = adenin-1-yl; R″ = H
7 R′ = adenin-1-yl; R″ = COPh
8 R′ = cytosin-1-yl; R″ = H
9 R′ = cytosin-1-yl; R″ = COPh

Each nucleoside trialcohol was isolated by a different procedure. The uracilyl derivative **4** was purified by application of ion-exchange chromatography. The adeninyl derivative (**6**) was converted into a picrate salt, which was purified by recrystallization. Regeneration of pure **6** was accomplished with an anion-exchange resin in the carbonate form[3]. In contrast to **6**, it was not possible to form a stable, crystalline picrate of the cytosinyl derivative (**8**). The general methodology, useful in the isolation of **4**, gave only very small amounts of **8**. Therefore, the crude nucleoside (**8**) was isolated as the organic soluble acetate and was washed free of salts. Removal of the acetate groups and column chromatography yielded pure **8**. In no case was it possible to achieve crystallization of any of the nucleoside trialcohols from common solvents. They were finally obtained as hygroscopic powders after lyophilization, and it was necessary to further dry these powders under high vacuum at elevated temperature.

The nucleoside trialcohols gave highly characteristic crystalline derivatives. Compounds **6** and **8** gave tribenzoates after purification *via* the picrate salts. Benzoylation of **4** did not give a crystalline derivative; therefore, a tri-*p*-nitrobenzoate (**5**) was characterized.

EXPERIMENTAL

Melting points were determined on a Kofler micro hot-stage and are corrected. Elementary analyses were determined by the Baron Consulting Co., Orange, Connecticut. T.l.c. was performed on Brinkmann F_{254} silica gel plates, and spots were located with a Mineralight lamp. The following solvent systems were used: (*A*) 5% aqueous disodium hydrogen phosphate; (*B*) 86:14 butyl alcohol–water; (*C*) 6:3:1 2-propanol–conc. ammonium hydroxide–water; (*D*) 9:1 chloroform–methanol; and (*E*) 9:1 ethyl acetate–methanol (all proportions v/v). Evaporations were performed *in vacuo* in a rotary evaporator with a bath temperature of 40–50°.

1-(1,3-Dihydroxy-2-propyl)-1-uracil-1-yl-1(R),*2-ethanediol*(**4**). — To a mixture of uridine (**1**, 2.5 g) and water (45 ml) was added sodium periodate (2.45 g) in small portions, while maintaining the temperature at 20–25° with an ice-bath. The reaction mixture was kept in a refrigerator, overnight, and poured into abs. ethanol (150 ml). After stirring for 15 min, the salt was removed by filtration and the filtrate was evaporated to dryness. The residue was dissolved in water (500 ml) and the solution was added, dropwise, to a stirred solution, protected from light[4], containing sodium borohydride (2 g) dissolved in water (50 ml). After the solution had been kept for an additional 2 h in the dark, the pH was adjusted to 7 with Amberlite IR-120 (H^+) resin. The resin was removed by filtration, and the water was evaporated to yield a residue which was dissolved in water. The solution was passed through an Amberlite IRC-50 (H^+) column, which was washed with water. The water was evaporated, and four 100-ml portions of methanol were added and evaporated to remove boric acid as methyl borate. The syrupy residue was dissolved in 30% aqueous methanol (20 ml), and the solution was applied to the top of a column of Dowex 1-X2 (OH^-, 200-400 mesh, 32 × 2.5 cm) which had been equilibrated with the same solvent[5]. The column was washed with 60% aqueous methanol (1200 ml), 90% aqueous methanol (2600 ml), and water (400 ml), and the product was eluted with 0.1M ammonium hydrogen carbonate solution. Fractions (8.5 ml) were collected, and the contents of tubes 97–110 were combined and evaporated to dryness. The residue was dissolved in water and lyophilized. The white solid was extremely hygroscopic and became gummy in a few minutes. Further drying in a drying pistol over phosporus pentoxide for 18 h at 100° gave 1.4 g, $[\alpha]_D^{23}+49\pm1°$ (*c* 1.7, water); $\lambda_{max}^{H_2O}$ 262 nm (ε 10,050) $\lambda_{min}^{H_2O}$ 230 nm (ε 1,930); i.r. data: 3350 (broad OH, C=NH), 1680 (-NHCO- of pyrimidinone), 1458 (uracil ring), 1112–1040 (plateau C-O, C-O-C), 812, and 782–760 cm^{-1} (pyrimidine CH); t.l.c.: R_F 0.75 (*A*), 0.24 (*B*) and 0.61 (*C*).

Anal. Calc. for $C_9H_{14}N_2O_6$: C, 43.90; H, 5.73; N, 11.38. Found: C, 43.88; H, 5.78; N, 11.40.

*Tri-*p*-nitrobenzoate* (**5**) *of* **4**. — Compound **4** (118 mg) was treated with

p-nitrobenzoyl chloride in pyridine for 25 h, and the mixture was finally heated on a steam-bath for 35 min. After the usual work-up, a syrup was obtained which solidified after standing for several h. The solid was triturated with methanol–chloroform, filtered off, and recrystallized from 1:1 acetone–methanol (12 ml). The tan-colored rosettes (252 mg) had m.p. 186–188°; i.r. data: 3480, 3060 (C=N–H), 1755, 1725 (*para*-benzenoid substitution), 1710 (benzoate C=O), (-NHCO- of pyrimidinone), 1600 (Ph and pyrimidine ring), 1520 (NO_2), 1345 (aromatic C–N), 1320 (NO_2), 1265 (benzoate C–O–C), 1102, 1092, 1078 (C–O, C–O–C), and 814 cm^{-1} (*para*-disubstituted Ph C–H); t.l.c.: R_F 0.70 (*D*) and (*E*).

Anal. Calc. for $C_{30}H_{23}N_5O_{15}$: C, 51.95; H, 3.34; N, 10.10. Found: C, 52.53; H, 3.42; N, 9.82.

1-(1,3-Dihydroxy-2-propyl)-1-(adenin-1-yl)-1(R),*2-ethanediol* (**6**). — Adenosine (**2**, 5.34 g) was suspended in water (80 ml), and sodium periodate (4.4 g) was added in small portions as described for **1**. The mixture was kept for 1.5 h at room temperature and then reduced as described for the preparation of **4**. After neutralization and filtration, the filtrate was evaporated to a small volume and adjusted with water to 250 ml.

A 100-ml aliquot from this solution was evaporated to dryness. Warm methanol (100 ml) was added to dissolve the residue, the solution was cooled to room temperature, 10% methanolic picric acid (200 ml) was added, and the solution was kept overnight at 0°. The yellow crystals (1.45 g) of the picrate were filtered off. A portion of the picrate (0.7 g) was recrystallized from methanol as tiny platelets (0.55 g), subliming at temperatures above 170° to form tiny needles, which decomposed and melted slowly with continued heating; $[\alpha]_D^{24}$ +43.0 ±0.4° (*c* 1.41, *N*,*N*-dimethylformamide); i.r. data: 3310–3110 (broad OH, NH), 1690 (protonated adenine ring), 1600 (Ph and purine ring), 1560, 1540 (NO_2, asymmetrical stretching), 1358 (aromatic C–N stretching), 1315 (NO_2, symmetrical stretching), 1115, and 1075–1040 cm^{-1} (C–O, C–O–C).

Anal. Calc. for $C_{16}H_{18}N_8O_{11}$: C, 38.54; H, 3.64; N, 22.50 Found: C, 38.56; H, 3.63; N, 22.80.

A sample of the picrate (760 mg) was suspended in water (350 ml). Bio-Rad AG1-X8 (CO_3^{2-}) ion-exchange resin was added in small portions with stirring until the yellow color had disappeared from the solution[3]. After an additional 1.5 h of stirring, the resin was removed by filtration. Evaporation of the water yielded a white foam, whereas lyophilization of an aqueous solution gave a white powder (345 mg), which was thoroughly dried *in vacuo* for 24 h at 65°; $[\alpha]_D^{23}$ +58.9 ±0.5° (*c* 1.63, water); $\lambda_{max}^{H_2O}$ 260 nm (ε 14,000); i.r. data: 3340 (broad OH, NH), 1638, 1592, 1570, 1475 (NH_2–C=N, purine ring), 1112, and 1040 cm^{-1} (plateau C–O); t.l.c.: R_F 0.88 (A), 0.29 (B), and 0.48 (C).

Anal. Calc. for $C_{10}H_{15}N_5O_4$: C, 44.61; H, 5.62; N, 26.01. Found: C, 44.45; H, 5.37; N, 26.09.

Tribenzoate (**7**) *of* **6** .— Compound **6** obtained from 0.7 g of the picrate was benzoylated with an excess of benzoyl chloride in pyridine. The viscous syrup,

obtained after the usual work-up, was dissolved in hot ethanol, decolorized with Norit A, and the volume was condensed to about 25 ml. To this solution was added 10% ethanolic picric acid (20 ml), and the mixture was heated at reflux for 1 h[6], and kept for 24 h at room temperature. The yellow crystals of the picrate were filtered off and recrystallized from methanol to yield 198 mg, m.p. 147-152°, with prior softening starting about 135°; i.r. data: 3080–3020 (NH), 1715 (benzoate C = O), 1690 (protonated adenine), 1605, 1578 (Ph and purine ring), 1545 (NO_2), 1360 (aromatic C–N), 1312 (NO_2), 1265 (benzoate C–O–C), 1105, 1093, 1065 (C–O, C–O–C), and 708 cm^{-1} (monosubstituted Ph).

Anal. Calc. for $C_{37}H_{30}N_8O_{14}$: C, 54.82; H, 3.73; N, 13.82. Found: C, 54.09; H, 3.72; N, 14.10.

To a solution of the picrate (188 mg) in acetone (40 ml) and water (10 ml) was added Bio-Rad AG1-X8 (CO_3^{2-}) resin in small portions until the yellow color had disappeared and the mixture was stirred for 1 h[7]. The resin was filtered off, and the solution was evaporated to a syrup, which was dried by several additions of ethanol followed by evaporation. The product was crystallized from methanol as clusters of needles (50 mg), m.p. 145–145.5°; i.r. data: 3280, 3080 (NH), 1715 (benzoate C=O), 1665 (NH_2–C = N), 1598, 1570 (Ph and purine ring), 1265 (benzoate C–O–C), 1115, 1082, 1065 (C–O, C–O–C), and 704 cm^{-1} (monosubstituted Ph); t.l.c.: R_F 0.60 (*D*) and 0.51 (*E*).

Anal. Calc. for $C_{31}H_{27}N_5O_7$: C, 64.01; H, 4.68; N, 12.03. Found: C, 64.37; H, 4.79; N, 12.21.

1-(1,3-Dihydroxy-2-propyl)-1-(cytosin-1-yl)-1(R),2-ethanediol (**8**). — Cytidine (**3**, 2.43 g) was dissolved in water (40 ml) and treated with sodium periodate (2.64 g) and sodium borohydride in the same manner as described for the preparation of **6**. After neutralization, filtration, and evaporation, three additions of ethanol and one of dry pyridine to the residue were followed by evaporation. The white solid was suspended in dry pyridine (50 ml) by trituration and treated with acetic anhydride (25 ml) for 26 h. After work-up, a syrup (1.91 g) was obtained; i.r. data: 3200–3000 (NH), 1740 (acetate C=O), 1660, 1620 (pyrimidine ring), 1230 (acetate C–O–C), 1125–1088, and 1050 cm^{-1} (C–O). The blocking groups were removed with methanolic ammonia (130 ml) that had been previously saturated at 0°. The residue was dissolved in a small amount of water and applied to a column of Dowex-1 X2 (OH^-, 200–400 mesh, 30 × 2 cm) resin[5]. The column was eluted with water and 12-ml fractions were collected. Fractions 26-40, which showed strong absorption at 270 nm, were combined and evaporated to a hard, clear syrup. Its solution in water was lyophilized, and the white powder was dried under high vacuum for 16 h at 65°, then for 1.5 h at 100° to give a hard, white hygroscopic glass (0.615 g); $[\alpha]_D^{22}$ +62 ±1° (*c* 1.6, water); $\lambda_{max}^{H_2O}$ 217 nm (ε 8,320), $\lambda_{min}^{H_2O}$ 250 nm (ε 5,600); i.r. data: 3340–3180 (broad OH, NH), 1675 (NH_2–C=N), 1635, 1610 (pyrimidine ring), 1115, 1060–1030 (C–O, C–O–C), and 778 cm^{-1} (pyrimidine CH); t.l.c.: R_F 0.78 (*A*), 0.13 (*B*), and 0.50 (*C*).

Anal. Calc. for $C_9H_{15}N_3O_5$:C, 44.08; H, 6.18; N, 17.13. Found; C, 44.25; H, 6.21; N, 17.06.

Tribenzoate (**9**) *of* **8**. — Compound **8** (96 mg) was benzoylated in a manner similar to the preparation of **7**. The gummy residue was dissolved in hot ethanol (5 ml). A solution of 5% ethanolic picric acid (10 ml) was added, and the mixture was heated at reflux for 1 h[6]. The picrate crystallized slowly overnight. Recrystallization from methanol gave fine, yellow needles (100 mg), m.p. 105–118°, and after recrystallization from methanol (10 ml) 76 mg, m.p. 105–107° (moistening at 102°). The elementary analysis and i.r. spectrum indicated a solvate containing 2 moles of methanol; i.r. data: 3380, 3060 (OH, NH), 1720 (benzoate C=O), 1680 (protonated cytosine), 1604, 1575 (Ph and pyrimidine ring), 1535 (NO_2), 1318 (NO_2), 1265 (benzoate C–O–C), 1115, 1100, 1078, 1070 (C–O, C–O–C), and 710 cm^{-1} (monosubstituted Ph).

Anal. Calc. for $C_{36}H_{30}N_6O_{15}\cdot 2\ CH_3OH$: C, 53.65; H, 4.47; N, 9.88. Found: C, 53.83; H, 4.22; N, 10.28.

The picrate (69 mg) was dissolved in 80% aqueous acetone (88 ml) and **9** was regenerated as described for the preparation of **7**. The product was crystallized and recrystallized from ethanol, giving clusters of large, feathery needles, m.p. 191–191.5°; i.r. data: 3280 (NH), 1720 (benzoate C=O), 1660 (NH_2–C=N), 1625, 1600sh (Ph and pyrimidine ring), 1265 (benzoate C–O–C), 795, 782 (pyrimidine CH), and 705 cm^{-1} (monosubstituted Ph); t.l.c.: R_F 0.35 (*D*) and 0.28 (*E*).

Anal. Calc. for $C_{30}H_{27}N_3O_8$: C, 64.62; H, 4.89; N, 7.54. Found: C, 64.18; H, 4.95; N, 7.33.

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