NUCLEIC ACID COMPONENTS AND THEIR ANALOGUES. CLII.*

SYNTHESIS OF 3-(β-D-RIBOFURANOSYL)-5,7-DIHYDROXY-1*H*-PYRAZOLO[4,3-*d*]PYRIMIDINE (OXOFORMYCIN)**

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1,3-Cycloaddition of 2,3,5-tri-O-benzyl-β-D-ribofuranosyldiazomethane to dimethyl acetylenedicarboxylate afforded the pyrazole derivative *V*, the ammonolysis of which led to the amide *VI*. When treated with hydrazine hydrate, the amide *VI* afforded the hydrazide *VII* and a small amount of the dihydrazide *VIII*. The acid-catalysed cyclisation of the dihydrazide *VIII* led to the 1*H*-pyrazolo[3,4-*d*]pyridazine derivative *IXb*, the benzyl groups of which were removed by hydrogenolysis to afford the C-nucleoside *IXa*. When heated in tert-butyl alcohol, the azide *X* (obtained from the hydrazide *VII*) afforded the 1*H*-pyrazolo[4,3-*d*]pyrimidine derivative *Ib*, the debenzylation of which afforded the C-nucleoside *Ia*.

In connection with a program on the synthesis of C-nucleosides we have worked out a method[1] for the synthesis of 3-substituted derivatives of 5,7-dihydroxy-1*H*-pyrazolo[4,3-*d*]pyrimidine as an approach to the synthesis of nucleosidic antibiotics[2] formycin and formycin B.*** In the present paper we wish to describe an application of this method to the synthesis of 3-(β-D-ribofuranosyl)-5,7-dihydroxy-1*H*-pyrazolo [4,3-*d*]pyrimidine (*Ia*) which had been obtained some time ago[3] in a low yield by Japanese authors on the biological oxidation of formycin and named oxoformycin. Compound *Ia* appeared as a versatile intermediate for the synthesis of structurally modified formycins. Only a few modified formycins have been hitherto prepared[4] with the use of formycin B obtained by a fermentative route.

The synthesis of compound *Ia* was started from the previously reported[5] 3,4,6-tri-O-benzyl-1-deoxy-1-ureido-2,5-anhydro-D-allitol (*II*) which was converted by nitrosation according to Kirmse[6] into 3,4,6-tri-O-benzyl-1-deoxy-1-(N-nitrosoureido)-2,5-anhydro-D-allitol (*III*). When treated with aqueous potassium hydroxide, the ethereal solution of the nitroso compound *III* afforded a solution of 2,3,5-tri-O-ben-

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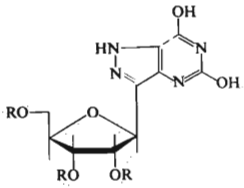
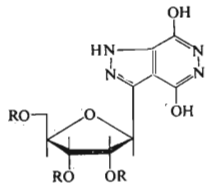
*** When the preliminary communication of the present results appeared, Goodman and coworkers[7] reported a synthesis of formycin B based on the preparation of 1*H*-pyrazolo[4,3-*d*]pyrimidine 3-substituted derivatives which had been worked out in our Laboratory.

zyl-β-D-ribofuranosyldiazomethane (*IV*) the reaction of which with dimethyl acetylenedicarboxylate resulted in a good yield of dimethyl 3-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl)pyrazole-4,5-dicarboxylate (*V*). By the action of methanolic ammonia at room temperature, compound *V* was converted into methyl 3-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl)-5-carbamoylpyrazole-4-carboxylate (*VI*) the structure of which was proposed by analogy to the previously reported course of ammonolysis of dialkyl pyrazole-4,5-dicarboxylates[1,8]. By the action of hydrazine hydrate, compound *VI* afforded a mixture of the hydrazide of 3-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl)-5-carbamoylpyrazole-4-carboxylic acid (*VII*) and about 10% of the dihydrazide of 3-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl)pyrazole-4,5-dicarboxylic acid (*VIII*). For the sake of comparison, the dihydrazide *VIII* was prepared also by hydrazinolysis of the dimethyl ester *V*. Cyclisation [9,10] of the dihydrazide *VIII* with 0·1M-HCl afforded 3-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl)-4,7-dihydroxy-1*H*-pyrazolo[3,4-*d*]-pyridazine (*IXb*) the benzyl groups of which were removed by hydrogenolysis to afford the crystalline 3-(β-D-ribofuranosyl)-4,7-dihydroxy-1*H*-pyrazolo[3,4-*d*]pyridazine (*IXa*).

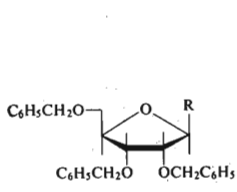
By the action of nitrous acid in aqueous dimethylformamide, the hydrazide *VII*

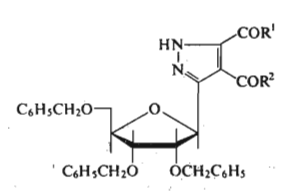


Ia, R = H
Ib, R = $CH_2C_6H_5$



IXa, R = H
IXb, R = $CH_2C_6H_5$





II, R = $CH_2NHCONH_2$
III, R = $CH_2\overset{\overset{NO}{|}}{N}CONH_2$
IV, R = CHN_2

V, $R^1 = R^2 = OCH_3$
VI, $R^1 = NH_2$, $R^2 = OCH_3$
VII, $R^1 = NH_2$, $R^2 = NHNH_2$

VIII, $R^1 = R^2 = NHNH_2$
X, $R^1 = NH_2$, $R^2 = N_3$

was converted to the azide of 3-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl)-5-carbamoylpyrazole-4-carboxylic acid (*X*) which was then heated in tert-butyl alcohol to afford 3-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl)-5,7-dihydroxy-1*H*-pyrazolo[4,3-*d*]pyrimidine (*Ib*) as the single product. The mass spectrum of compound *Ib* shows an intensive peak (B + 30) corresponding to the aglycone carrying a protonated formyl group arisen by fragmentation of the sugar residue. According to Robbins[11], this fragmentation is characteristic of C-nucleosides. The benzyl groups of compound *Ib* were removed with the use of sodium in liquid ammonia[12] under the formation of the desired nucleoside *Ia* the ultraviolet and infrared spectrum of which is in accordance with the data of oxoformycin[3].

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block) and are uncorrected. Analytical samples were dried at 25°C/0·05 Torr for 10 h. UV spectra were recorded on an Optica Milano Model CF-4 apparatus. IR spectra were measured on a Zeiss Model UR-10 apparatus. ORD spectra were taken on a Jasco Model ORD/UV-5 spectropolarimeter. NMR spectrum was measured on a Varian HA-100 apparatus at 100 MHz. The mass spectrum was measured on a MS-902 mass spectrometer.

3,4,6-Tri-O-benzyl-1-deoxy-1-(N-nitrosoureido)-2,5-anhydro-D-allitol (*III*)

A suspension of 3,4,6-tri-O-benzyl-1-deoxy-1-ureido-2,5-anhydro-D-allitol (*II*; 4·75 g; 0·01 mol) in ether (50 ml) was treated dropwise over 5 min at −30°C with a solution of nitrogen dioxide (1·5 g; 0·016 mol) in ether (20 ml), the mixture diluted with ether (150 ml), and poured into a mixture of ice and water. The ethereal layer was separated, washed with three 50 ml portions of saturated aqueous sodium hydrogen carbonate, dried over sodium sulfate, and evaporated (bath temperature 35°C). The residue was chromatographed on a 3·5 × 45 cm column of silica gel in the solvent system benzene–ethyl acetate (4 : 1). The ultraviolet-absorbing fraction was evaporated under diminished pressure and dried at 25°C/0·1 Torr for 8 h. Yield, 3·84 g (76%) of compound *III* in the form of a sirup which may be stored at 0°C for several days without any decomposition. UV spectrum (in ethanol): λ_{max} 213 and 236 nm (log ε 4·17 and 3·66, resp.). IR spectrum (in CCl_4): 1 743 cm^{-1} (C=O), 3 530 cm^{-1} and 3410 cm^{-1} (NH_2), 1 500 cm^{-1} (NO). For $C_{28}H_{31}N_3O_6$ (505·55) calculated: 66·52% C, 6·18% H, 8·31% N; found: 66·59% C, 6·21% H, 8·00% N.

Dimethyl 3-(2,3,5-Tri-O-benzyl-β-D-ribofuranosyl)pyrazole-4,5-dicarboxylate (*V*)

A precooled (0°C) solution of compound *III* (2·53 g; 0·005 mol) in ether (80 ml) was shaken for 5 min with a precooled (0°C) 30% aqueous KOH (50 ml). The ethereal layer was separated and dried at 0°C over potassium hydroxide for 5 min. The yellow ethereal solution of diazo compound *IV* was filtered and the filtrate treated with a solution of dimethyl acetylenedicarboxylate[13] (0·80 g; 0·056 mol) in ether (15 ml). When the yellow color of the mixture disappeared, the mixture was evaporated to dryness under diminished pressure and the residual sirup chromatographed on a 2 × 30 cm column of silica gel. The column was washed with 9 : 1 benzene–ethyl acetate and the product eluted with 4 : 1 benzene–ethyl acetate. The eluate was evaporated under diminished pressure, the residual sirup coevaporated with methanol (50 ml) at 35°C/15 Torr, and the final residue dried at 25°C/0·1 Torr for 6 h. Yield, 1·74 g (59%) of compound *V* in the form of a sirup, $[\alpha]_D^{25}$ +110·4° (*c* 0·51; chloroform). UV spectrum (in ethanol); λ_{max} 213 nm (log ε 4·31). IR spectrum (in tetrachloromethane): 1 741 cm^{-1} and 1 746 cm^{-1} (C=O), 3420

cm^{-1} (NH assoc.). For $C_{33}H_{34}N_2O_8$ (586·6) calculated: 67·56% C, 5·84% H, 4·78% N; found: 67·82% C, 5·90% H, 4·59% N.

Methyl 3-(2,3,5-Tri-O-benzyl-β-D-ribofuranosyl)-5-carbamoylpyrazole-4-carboxylate (*VI*)

Compound *V* (2·93 g; 0·005 mol) was dissolved in 20 ml of methanol presaturated at 20°C with ammonia gas. The solution was allowed to stand at room temperature for two days, evaporated under diminished pressure, and the residual sirup chromatographed on a 2 × 30 cm column of silica gel in the solvent system 1 : 1 benzene–ethyl acetate. The product was dissolved in methanol (30 ml), the solution evaporated at 35°C/15 Torr, and the residue dried at 25°C/0·1 Torr. Yield, 2·60 g (91%) of compound *VI* in the form of a sirup, $[\alpha]_D^{25}$ +71·3° (*c* 0·50; chloroform). UV spectrum (in ethanol): λ_{max} 216 and 300 nm (log ε 4·22 and 2·73 resp.). IR spectrum (in CCl_4): 1693 cm^{-1} (C=O) ester, 1679 cm^{-1} (C=O amide). For $C_{32}H_{33}N_3O_7$ (571·6) calculated: 67·23% C, 5·82% H, 7·35% N; found: 67·10% C, 5·87% H, 7·35% N.

Hydrazide of 3-(2,3,5-Tri-O-benzyl-β-D-ribofuranosyl)-5-carbamoylpyrazole-4-carboxylic Acid (*VII*)

A mixture of compound *VI* (2·86 g; 0·005 mol), ethanol (20 ml), and 80% hydrazine hydrate (2·0 ml) was refluxed for 45 min, evaporated under diminished pressure, and the residue coevaporated under the same conditions with three 15 ml portions of ethanol. The residual sirup (containing compound *VII* along with 10% of the dihydrazide *VIII*) was chromatographed on a 3·5 × × 40 cm column of silica gel in the solvent system 2 : 3 benzene–ethyl acetate. The sirupous chromatographically homogeneous fraction of compound *VII* was coevaporated with 20 ml of methanol and then dried at 25°C/0·1 Torr. Yield, 2·4 g (84%) of compound *VII* in the form of a sirup, $[\alpha]_D^{25}$ +178·2° (*c* 0·51; chloroform). UV spectrum (in ethanol): λ_{max} 214 nm (log ε 4·40). IR spectrum (in CCl_4): 1679 cm^{-1} (C=O amide), 1642 cm^{-1} (C=O hydrazide), 3470 cm^{-1} (NH pyrazole). For $C_{31}H_{33}N_5O_6$ (571·6) calculated: 65·13% C, 5·82% H, 12·25% N; found: 65·24% C, 6·04% H, 11·98% N.

Dihydrazide of 3-(2,3,5-Tri-O-benzyl-β-D-ribofuranosyl)pyrazole-4,5-dicarboxylic Acid (*VIII*)

A solution of compound *V* (2·93 g; 0·005 mol) in ethanol (20 ml) was treated with 80% hydrazine hydrate (1·0 ml), the mixture refluxed for 5 hours, and allowed to cool down to deposit a solid which was recrystallised from ethanol. Yield, 2·37 g (81%) of compound *VIII*, m.p. 158–159·5°C (ethanol); $[\alpha]_D^{25}$ +183·4° (*c* 0·50; chloroform). UV spectrum (in ethanol): λ_{max} 214 nm (log ε 4·39). IR spectrum (in chloroform): 1642 cm^{-1} and 1664 cm^{-1}(C=O hydrazide), 3420 cm^{-1} (NH pyrazole). For $C_{31}H_{34}N_6O_6$ (586·6) calculated: 63·47% C, 5·84% H, 14·33% N; found: 63·28% C, 5·47% H, 14·24% N.

3-(2,3,5-Tri-O-benzyl-β-D-ribofuranosyl)-4,7-dihydroxy-1*H*-pyrazolo[3,4-*d*]pyridazine (*IXb*)

A solution of compound *VIII* (2·93 g; 0·005 mol) in 70% aqueous ethanol (25 ml) was treated with concentrated hydrochloric acid (0·3 ml), the mixture refluxed for 4 hours, and evaporated under diminished pressure. The residue was chromatographed on a 3·5 × 30 cm column of silica gel, the column washed with 500 ml of 1 : 1 benzene–ethyl acetate, the product eluted with 1 : 2 benzene–ethyl acetate, the eluate evaporated, and the residue dried at 25°C/0·1 Torr for 6 hours. Yield, 2·25 g (81%) of compound *IXb* in the form of a sirup, $[\alpha]_D^{25}$ +173·0° (*c* 0·48; chloroform). IR spectrum (in CCl_4): 1652 cm^{-1} (C=O). UV spectrum (in ethanol): λ_{max} 213 nm and 265 nm

(log ε 4·36 and 3·74). For $C_{31}H_{30}N_4O_6$ (554·6) calculated: 67·13% C, 5·45% H, 10·10% N; found: 66·91% C, 5·51% H, 10·13% N.

3-(β-D-Ribofuranosyl)-4,7-dihydroxy-1*H*-pyrazolo[3,4-*d*]pyridazine (*IXa*)

Compound *IXb* (1·11 g; 0·002 mol) was hydrogenated at room temperature in a pressureless apparatus over 0·5 g of a 5% palladium on barium sulfate catalyst in 25 ml of 96% ethanol containing one drop of concentrated hydrochloric acid until the hydrogen uptake ceased for 2½ h). Ethanol (20 ml) was then added, the mixture heated to boil, and filtered while hot to remove the catalyst. The filtrate was concentrated to the volume of 5—10 ml and allowed to crystallize for several hours. Yield, 0·55 g (96%) of compound *IXa*, m.p. 255—256° (decomp.) (90% aqueous ethanol); $[\alpha]_D^{25}$ +14·4° (*c* 0·48; water). UV spectrum, in 0·1M-HCl: λ_{max} 266 nm (log ε 3·75); in 0·05M-NaOH: λ_{max} 216 and 276 nm (log ε 4·29 and and 3·85, resp.). NMR spectrum (in hexadeuteriodimethyl sulfoxide + 1% of CH_3CO_2D; tetramethylsilane as internal standard; δ in p.p.m.) 5·02 (d, $J_{1',2'}$ 7·0 Hz, $H_{1'}$); 4·28 (q, $J_{2',1'}$ 7·0 Hz, $J_{2',3'}$ 5·5 Hz, $H_{2'}$); 4·04 (q, $J_{3',2'}$ 5·5 Hz, $J_{3',4'}$ 3·5 Hz, $H_{3'}$); 3·88 (q, $J_{4',3'} = J_{4',5'a} = J_{4',5'b}$ 3·5 Hz, $H_{4'}$); 3·63 (q, $J_{5'a,4'}$ 3·5 Hz, $J_{gem.}$ 12·0 Hz, $H_{5'a}$); 3·46 (q, $J_{5'b,4'}$ 3·5 Hz, J_{gem} 12·0 Hz, $H_{5'b}$). For $C_{10}H_{12}N_4O_6$ (284·2) calculated: 42·25% C, 4·26% H, 19·71% N; found: 41·96% C, 4·41% H, 19·62% N.

3-(2,3,5-Tri-O-benzyl-β-D-ribofuranosyl)-5,7-dihydroxy-1*H*-pyrazolo[4,3-*d*]pyrimidine (*Ib*)

A solution of the hydrazide *VII* (350 mg; 6·1 mmol) in dimethylformamide (10 ml) was cooled down to 0°C and treated under stirring with 2M-HCl (1·0). After 3 minutes, 2M-$NaNO_2$ (1·0 ml) was added, the whole mixture kept in an ice bath for 2 hours, poured onto ice, and extracted with three 30 ml portions of ether. The ethereal extract was dried over sodium sulfate, evaporated under diminished pressure at 25°C, and the residual sirup dissolved in tert-butyl alcohol (20 ml). The solution was refluxed for 90 min, evaporated under diminished pressure, and the residue coevaporated with toluene (15 ml). The final residue was chromatographed on a 2 × 25 cm column of silica gel in the solvent system benzene–ethyl acetate (1 : 1). The crystalline, chromatographically homogeneous residue (142 mg; m.p. 188—190°C) was recrystallised from 96% ethanol. Yield, 93·2 mg (28%) of compound *Ib*, m.p. 192—193°C (96% ethanol); $[\alpha]_D^{25}$ −23·0° (*c* 0·20;) chloroform). UV spectrum (in ethanol): λ_{max} 288 nm (log ε 3·76). IR spectrum (in chloroform): 1694 cm^{-1} and 1710 cm^{-1} (C=O); 3385 cm^{-1} and 3430 cm^{-1} (NH). Mass spectrum: molecular peak at *m/e* 554 and intensive peak at *m/e* 181 (B + 30). For $C_{31}H_{30}N_4O_6$ (554·6) calculated: 67·13% C, 5·54% H, 10·10% N; found: 67·08% C, 5·49% H, 10·42% N.

3-(β-D-Ribofuranosyl)-5,7-dihydroxy-1*H*-pyrazolo[4,3-*d*]pyrimidine (*Ia*)

A gently refluxing (reflux condenser packed with dry ice) solution of compound *Ib* (46·1 mg) in 5 ml of liquid ammonia (freshly distilled over sodium) was treated portionwise with sodium wire in glass capillary (for the experimental technique see the methylation of saccharides[14]) until the blue colour persisted. Total uptake, 16·5 mg of sodium. The stiring was continued for additional 15 min, the blue color removed by the addition of one drop of methanol, and the ammonia evaporated first at atmospheric pressure and then at 15 Torr. The residue was dissolved in 3 ml of water and the solution applied to a 2 cm × 25 cm column of Dowex 50 (H^+) ion exchange resin. The column was eluted with water and the first 100 ml of the eluate was evaporated under diminished pressure to the volume of about 3 ml. The concentrate was kept at room temperature to deposit crystals (15·5 mg), m.p. 284—286°C (water). The mother liquors were chromatographed on Dowex 1 (acetate) ion exchange resin; elution with 5% aqueous acetic acid

afforded additional 3·5 mg of compound *Ia* of the above purity (overall yield, 81%. ORD spectrum (in water): $[\Phi]_{262}$ +5330°, $[\Phi]_{310}$ −2010°. UV spectrum, in 0·1M-HCl: λ_{max} 288 nm (log ε 3·76); in 0·05M-NaOH: λ_{max} 226 nm, 250 nm, and 303 nm (log ε 4·43, 3·81, and 3·72, resp.). IR spectrum in KBr is identical with that of oxoformycin prepared by Umezawa[3]. For $C_{10}H_{12}N_4O_6$ (284·2) calculated: 42·25% C, 4·26% H, 19·71% N; found: 42·10% C, 4·19% H, 19·88% N.

REFERENCES

1. Sprinzl M., Farkaš J., Šorm F.: Tetrahedron Letters *1969*, 289.
2. Suhadolnik R. J.: *Nucleoside Antibiotics*, p. 354. Wiley-Interscience, New York 1970.
3. Ishizuka M., Sawa T., Koyama G., Takeuchi T., Umezawa H.: J. Antibiotics (Tokyo) *21*, 1 (1968).
4. Watanabe S., Matsuhashi G., Fukatsu S., Koyama G., Maeda K., Umezawa H.: J. Antibiotics (Tokyo) Ser. *A 19*, 93 (1966).
5. Bobek M., Farkaš J.: This Journal *34*, 1684 (1969).
6. Kirmse W., Buschhoff M.: Chem. Ber. *100*, 1491 (1967).
7. Acton E. M., Ryan K. J., Henry D. W., Goodman L.: Chem. Commun. *1971*, 986.
8. Jones R. G., Whitehead C. W.: J. Org. Chem. *20*, 1342 (1955).
9. Patel N. R., Castle R. N.: J. Heterocycl. Chem. *3*, 512 (1966).
10. Jones R. G.: J. Am. Chem. Soc. *78*, 159 (1956).
11. Townsend L. B., Robins R. K.: J. Heterocycl,. Chem. *6*, 459 (1969).
12. Reist E. J., Bartuska V. J., Goodman L.: J. Org. Chem. *29*, 3725 (1964).
13. Huntress E. H., Lesslie T. E., Bornstein J.: Org. Syn. *32*, 55 (1952).
14. Isbell H. S., Frush H. L., Bruckner B. H., Kowkabany G. N., Wampler G.: Anal. Chem. *29*, 1523 (1957).

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