4-β-D-RIBOFURANOSYL-1,2,4-TRIAZIN-3,5(2*H*,4*H*)-DIONE AND 4-β-D-RIBOFURANOSYL-6-METHYL-1,2,4-TRIAZIN-3,5(2*H*,4*H*)-DIONE*

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Received November 10th, 1980

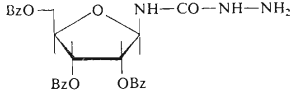
4-β-D-Ribofuranosyl-1,2,4-triazin-3,5(2*H*,4*H*)-dione (*IX*) and 4-β-D-ribofuranosyl-6-methyl-1,2,4-triazin-3,5(2*H*,4*H*)-dione (*XI*) were prepared by cyclization of (*Z*)-4-β-D-ribofuranosylsemicarbazones of methyl glyoxylate (*VI*) and methyl pyruvate (*VIII*) in the methanolic solution of sodium methoxide. A mixture of (*E*)- and (*Z*)-ribosylsemicarbazones *III* and *IV* was prepared by condensation of the ribosylsemicarbazide *I* with methyl dimethoxyacetate and a mixture of (*E*)- and (*Z*)-isomers *V* and *VIII* was obtained on condensation of *I* with methyl pyruvate. The (*Z*)-isomer *VI* was prepared on acid-catalyzed isomerisation of the (*E*)-isomer *III* while the (*Z*)-isomer *VIII* was obtained on the UV-irradiation of isomer *V*.

The earlier described ribosylations of the mercuric salt of 6-azauracil[1] and 5-methyl-6-azauracil[2] lead to a mixture of 1-ribosyl, 3-ribosyl, and 1,3-diribosyl derivatives of 6-azauracil. It followed from our earlier studies[3-5] that the appropriate ribosyl derivatives of glyoxylic acid semicarbazones could serve as a suitable starting material for an unambiguous preparation of the 3-ribosyl nucleosides. In the mentioned papers, the semicarbazones of glyoxylic acid were shown to be suitable intermediates for the preparation of 6-azauracil and its derivatives[3-5]. The isomerisation, alkylation, ribosylation, and cyclization of semicarbazones and thiosemicarbazones[6] of glyoxylic acid esters was also followed. The ribosylation led to various products according to the method used. The silyl method[5] afforded the 2-β-ribosyl derivative while the ribosylation of the semicarbazone salts[4] led to a mixture of 2-β-, 4-β-, and 4-α-ribosyl derivatives, in the dependence on the used salt. A considerably easier cyclization[3] of the (*Z*)-isomer to the corresponding 6-azauracil derivatives, in comparison with the (*E*)-isomer, was also demonstrated.

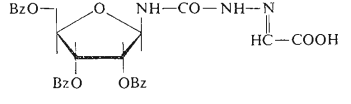
On the basis of these findings we now used the cyclization of the appropriate 4-ribosylsemicarbazones of glyoxylic acid and pyruvic acid for an unambiguous preparation of the 3-ribosyl derivatives *IX* and *XI*. The starting 4-β-D-ribosylsemicarbazones were prepared on condensation of the ribosylsemicarbazide *I* with methyl

* Part XXXIII in the series Analogues of Nucleosides; Part XXXII: Nucleic Acids Res. *8*, 6233 (1980).

dimethoxyacetate and methyl pyruvate. The semicarbazone *III* was also prepared on condensation of semicarbazide *I* with glyoxylic acid and on subsequent esterification of the acid *II* with diazomethane. The total yield of this procedure is considerably lower, however, than the yield of the condensation of *I* with methyl dimethoxyacetate. The reaction of semicarbazide *I* with methyl dimethoxyacetate proceeded in refluxing dichloromethane within 5 h. A 6·4 : 1 mixture of (*E*)-isomer *III* and (*Z*)-isomer *VI* was obtained. The isomers were separated by column chromatography on silica gel. The (*E*)-isomer *III* underwent an isomerisation when heated in the solution of hydrogen chloride in toluene. The reaction mixture afforded, on chromatography on silica gel, 21% of the (*Z*)-isomer and 74% of the (*E*)-isomer. The semicarbazone of methyl pyruvate *V* was prepared by the reaction of methyl pyruvate with semicarbazide *I* in 1,2-dichloroethane. Also in this case a small amount (5%) of the (*Z*)-isomer was isolated. Attempt at the acid-catalyzed isomerisation of the (*E*)-semicarbazone failed. Therefore, the phenylsemicarbazone *IV* was prepared as a model compound and used for the study of a possible UV-induced isomerisation[3,8]. A 0·1% solution of *IV* in methanol was irradiated by a medium-pressure mercury lamp for



I



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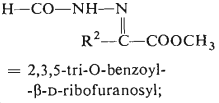
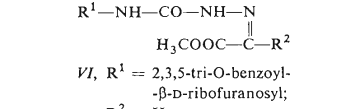
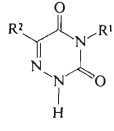


III, R^1 = 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl; R^2 = H
IV, $R^1 = C_6H_5$; $R^2 = CH_3$
V, R^1 = 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl; $R^2 = CH_3$



VI, R^1 = 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl; R^2 = H
VII, $R^1 = C_6H_5$; $R^2 = CH_3$
VIII, R^1 = 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl; $R^2 = CH_3$



IX, R^1 = β-D-ribofuranosyl; R^2 = H
X, $R^1 = C_6H_5$, $R^2 = CH_3$
XI, R^1 = β-D-ribofuranosyl; $R^2 = CH_3$

1 h. Work-up of the solution afforded the (*Z*)-isomer (44%) and the (*E*)-isomer (38%). The isomerisation of the ribosylsemicarbazone *V* is accompanied by side-reactions. A 30 min irradiation furnished the (*Z*)-isomer in a 36% yield and the (*E*)-isomer in a 28% yield.

The configuration on the N=C bond of particular isomers was unambiguously determined on the basis of IR spectra[4]. The stretching vibrations $\nu(N^2H)$ of the (*E*)-isomers *II*, *III*, *IV*, and *V* can be found at 3 347, 3 343, 3 370, and 3 366 cm^{-1} while in the spectra of the (*Z*)-isomers *VI*, *VII* and *VIII* appear at 3 292, 3 297, and 3 295 cm^{-1}. The γ(CH) band of N=CH group of the (*E*)-semicarbazone *III* is located at 915 cm^{-1}, in contrast to the (*Z*)-semicarbazone *VI*, where γ(CH) appear at 863 cm^{-1}. These values are in agreement with the data for the 4-substituted (*Z*)- and (*E*)-semicarbazones given in ref.[4]. Also the easy cyclization of the (*Z*)-isomers to the corresponding derivatives of 6-azauracil (0·1 M solution of sodium methoxide in methanol, room temperature) confirms the assigned configuration, in analogy with the earlier findings[3]. During the cyclization of the benzoylated ribosylsemicarbazones *VI* and *VIII* a simultaneous debenzoylation takes place under formation of the free ribosyl derivatives of 6-azauracil *IX* and *XI*.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block). The infrared spectra were recorded on a UR-20 (Carl Zeiss, Jena) apparatus. Optical rotations were taken on an automatic Perkin-Elmer 141 MC polarimeter. Column chromatography was performed on the Pitra silica gel (particle size, 30–60 μ; Service Laboratories of this Institute). Photochemical reactions were performed with a 125 W medium-pressure mercury lamp in the quartz immersion well cooled with water.

Methyl Dimethoxyacetate

Glyoxylic acid (hydrate, 98%; Aldrich-Europe; 10 g) was co-distilled with toluene (0·5 l) *in vacuo*. The residue was dissolved in methanol (250 ml), concentrated sulfuric acid (1 ml) was added and the solution was refluxed for 5 h. After cooling down, the solution was shaken with anhydrous potassium carbonate (5 g). The mixture was evaporated to a 100 ml volume and benzene (200 ml) was added. The insoluble portion was filtered off and washed with benzene (50 ml). The combined filtrates were evaporated to a volume of c. 40 ml and the solution was removed from an oily residue which was washed with benzene (10 ml) once again. The combined benzene solutions were concentrated to a 12 ml volume and the residue was distilled under diminished pressure (2·3 kPa). The fraction boiling at 65–67°C was collected. Yield, 7·2 g (58%) of methyl dimethoxyacetate. IR spectrum of the obtained compound is identical with the reported one[9].

Glyoxylic Acid (E)-4-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)semicarbazone (*II*)

A mixture of semicarbazide[7] *I* (520 mg; 1 mmol), methanol (10 ml), and glyoxylic acid (hydrate, 98%; Aldrich-Europe; 0·1 g) was stirred for 30 min at room temperature and evaporated to a 5 ml volume. The compound deposited on a 2 h standing was filtered off and washed with methanol.

Yield, 560 mg (97%) of semicarbazone *II*, m.p. 208·5—210·5°C. $[\alpha]_D^{25}$ −75° (*c* 0·45; dimethylformamide). IR spectrum (KBr): 3 440, 3 360, 3 235, and sh 3 185 cm^{-1} (OH, NH), 1 728 cm^{-1} (C=O benzoate), 1 692 cm^{-1} (amide I), 1 601 and sh 1 589 cm^{-1} (ring benzoate + C=N), 1 534 and 1 541 cm^{-1} (amide II); saturated solution in $CHCl_3$: 3 347 cm^{-1} (NH). For $C_{29}H_{25}N_3O_{10}$ (575·5) calculated: 60·52% C, 4·38% H, 7·30% N; found: 60·45% C, 4·51% H, 7·44% N.

Methyl Glyoxylate (*E*)-4-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)semicarbazone (*III*)

A) Ethereal solution of diazomethane was dropped into a stirred mixture of methanol (60 ml) and the acid *II* (575 mg; 1 mmol) until the acid had dissolved. The reaction course was monitored by TLC in the system toluene–ethyl acetate (1 : 1). The solution was evaporated under diminished pressure and the residue was chromatographed on a silica gel column (150 g) in the system toluene–ethyl acetate (1 : 1). Crystallization of the residue of the main UV-absorbing fraction from acetone afforded 327 mg (55·5%) of methyl ester *III*, m.p. 184—186°C. Crystallization of the mother liquors residue furnished additional 88 mg (15%) of the same compound. $[\alpha]_D^{25}$ −88° (*c* 0·44; ethyl acetate). IR spectrum is identical with that of an authentic compound[4]. For $C_{30}H_{27}N_3O_{10}$ (589·5) calculated: 61·12% C, 4·62% H, 7·13% N; found: 61·06%C, 4·67% H, 7·21% N.

B) To a solution of semicarbazide *I* (520 mg; 1 mmol) in dichloromethane (10 ml) was added methyl dimethoxyacetate (200 mg). The solution was refluxed for 5 h, evaporated under diminished pressure, and the residue was chromatographed on a silica gel column (80 g) in the system chloroform–ethyl acetate (7 : 3). Yield, 413 mg (70%) of the (*E*)-isomer *III* and 64 mg (11%) of the (*Z*)-isomer *VI*.

Methyl Glyoxylate (*Z*)-4-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)semicarbazone (*VI*)

A solution of the (*E*)-isomer *III* (1·5 g) in 0·2M solution of hydrogen chloride in toluene (100 ml) was heated at 80°C for 30 min. After cooling down, toluene was evaporated under diminished pressure and the residue was chromatographed on a silica gel column (200 g) in the system chloroform–ethyl acetate (7 : 3). Yield, 1·11 g (74%) of the (*E*)-isomer *III* and 310 mg (21%) of the (*Z*)-isomer *VI*. $[\alpha]_D^{25}$ +30° (*c* 0·46; dimethylformamide). IR spectrum (chloroform, *c* 0·003 mol.l^{-1}): 3 412 cm^{-1} (N^4—H), 3 292 cm^{-1} (N^2—H); *c* 2%: 1 727 cm^{-1} (C=O benzoate, amide I of monomer), sh 1 695 and 1 710 cm^{-1} (C=O ester, amide I of dimer), 1 603 and 1 586 cm^{-1} (8a, C=N, 8b), sh 1 541, 1 523, sh 1 506, and sh 1 495 cm^{-1} (amide II), 1 453 cm^{-1} (19b), 1 441 cm^{-1} (CH_3), 1 268 cm^{-1} (C—O benzoate), 1 122 cm^{-1} (amide III), 863 cm^{-1} (N=C—H). For $C_{30}H_{27}N_3O_{10}$ (589·5) calculated: 61·12% C, 4·62% H, 7·13% N; found: 61·00% C, 4·63% H, 6·97% N.

Methyl Pyruvate (*E*)-4-Phenylsemicarbazone (*IV*)

Methyl pyruvate[10] (400 mg) was added to a solution of 4-phenylsemicarbazide (453 mg; 3 mmol) in methanol (6 ml). The deposited compound was filtered off after 3 h. Yield, 615 mg (87%) of *IV*, m.p. 180—182°C. Evaporation of the mother liquors and crystallization of the residue from methanol afforded additional 30 mg (4%) of the same compound. IR spectrum (chloroform, *c* 0·003 mol.l^{-1}): 3 382 and 3 370 cm^{-1} (NH); *c* 2%: 1 704 cm^{-1} (C=O, amide I), 1 598 cm^{-1} (C=N, ring), 1 542 cm^{-1} (amide II), sh 1 504 and 1 450 cm^{-1} (ring), sh 1 440 cm^{-1} (CH_3), 1 374 cm^{-1} (C—CH_3), 1 163 and 1 144 cm^{-1} (amide III). For $C_{11}H_{13}N_3O_3$ (235·2) calculated: 56·16% C, 5·57% H, 17·86% N; found: 56·06% C, 5·57% H, 18·08% N.

Methyl Pyruvate (*Z*)-4-Phenylsemicarbazone (*VII*)

A solution of *IV* (250 mg) in methanol (250 ml) was irradiated with the mercury lamp. The solution was evaporated under diminished pressure and the residue was chromatographed on a silica gel column (70 g) in the system toluene–ethyl acetate (5 : 2). On crystallization of the single fractions from 2-propanol, 95 mg (38%) of *IV* and 110 mg (44%) of *VII* (m.p. 132—133°C) were obtained. IR spectrum (chloroform, *c* 0·003 mol.l^{-1}): 3 392 cm^{-1} (N^4—H), sh 3 358 and 3 297 cm^{-1} (N^2—H); *c* 2%: sh 1 729, sh 1 703, and 1 693 cm^{-1} (C=O, amide I), 1 603 and 1 594 cm^{-1} (ring 8a, 8b + C=N); 1535 cm^{-1} (amide II), sh 1486 cm^{-1} and 1447 cm^{-1} (19b), sh 1 439, 1 417, and 1 377 cm^{-1} (CH_3), 1 304, 1 156, and 1 140 cm^{-1} (amide III). For $C_{11}H_{13}N_3O_3$ (235·2) calculated: 56·16% C, 5·57% H, 17·86% N; found: 56·34% C, 5·71% H, 18·04% N.

Methyl Pyruvate (*E*)-4-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)semicarbazone (*V*)

Methyl pyruvate (150 mg) was added to a solution of ribosylsemicarbazide[7] I (520 mg; 1 mmol) in 1,2-dichloroethane (6 ml). The whole was left to stand at room temperature for 4 h, the solution was evaporated under diminished pressure and the residue was chromatographed on a column of silica gel (60 g) in the system toluene–ethyl acetate (2 : 1). The (*Z*)-isomer *VIII* (30 mg; 5%) and the (*E*)-isomer *V* (551 mg; 91%) were obtained. For title compound, $[\alpha]_D^{25}$ −2° (*c* 0·49; ethyl acetate). IR spectrum (chloroform, *c* 0·003 mol.l^{-1}): 3 412 cm^{-1} (N^4—H), 3 366 cm^{-1} (N^2—H); *c* 2%: 1 726 cm^{-1} (C=O benzoate, amide I of monomer), sh 1 712 and sh 1 692 cm^{-1} (C=O ester, amide I of dimer), sh 1 616 cm^{-1} (C=N), 1 604 and 1 587 cm^{-1} (8a, 8b), sh 1 541, 1 532, and sh 1 521 cm^{-1} (amide II), 1 454 cm^{-1} (19b), 1 440 and 1 376 cm^{-1} (CH_3), sh 1 142 cm^{-1} (amide III). For $C_{31}H_{29}N_3O_{10}$ (603·6) calculated: 61·69% C, 4·84% H, 6·96% N; found: 61·89% C, 4·94% H, 6·82% N.

Methyl Pyruvate (*Z*)-4-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)semicarbazone (*VIII*)

A solution of *V* (250 mg) in methanol (250 ml) was irradiated with mercury lamp for 30 min. The solution was then evaporated under diminished pressure. Chromatography of the residue on a silica gel column (80 g) in the system toluene–ethyl acetate (5 : 2) afforded 70 mg (28%) of *V* (2nd fraction) and 89 mg (35·6%) of *VIII* (1st fraction). $[\alpha]_D^{25}$ −73% (*c* 0·49; ethyl acetate). IR spectrum (chloroform, *c* 0·003 mol.l^{-1}): 3 404 and 3 295 cm^{-1} (NH); *c* 2%: 1 728 cm^{-1} (C=O benzoate, amide I of monomer), sh 1 709 cm^{-1} (C=O ester, amide I of dimer), 1 604 and 1 587 cm^{-1} (8a, 8b, C=N), 1 529 cm^{-1} (amide II), 1 454 and 1 439 cm^{-1} (19b, CH_3), 1 379 cm^{-1} (CH_3), 1 126 cm^{-1} (amide III). For $C_{31}H_{29}N_3O_{10}$ (603·6) calculated: 61·69% C, 4·84% H, 6·96% N; found: 61·68% C, 4·90% H, 6·85% N.

4-(β-D-Ribofuranosyl)-1,2,4-triazin-3,5(2*H*,4*H*)-dione (*IX*)

A solution of the (*Z*)-semicarbazone *III* (295 mg; 0·5 mmol) in 0·1M methanolic solution of sodium methoxide (15 ml) was left to stand for 1 h at room temperature and then it was neutralized with Dowex 50 (H^+; pre-washed with methanol). The resin was filtered off, washed with methanol (30 ml), and the combined filtrates were evaporated under diminished pressure. Crystallization of the residue from ethanol afforded 74 mg (60%) of *V*, m.p. 187—188·5°C. Mother liquors furnished additional 10 mg (8%) of the same compound. $[\alpha]_D^{25}$ −28° (*c* 0·39; water). UV spectrum — 0·1M-HCl: λ_{max} 264 nm (log ε 3·70), λ_{min} 221 nm (log ε 3.28); H_2O: λ_{inax} 263 nm (log ε 3·73), λ_{min} 222 nm (log ε 3·42); 0·1M-NaOH: λ_{max} 250 and 303 nm (log ε 4·05 and 3·40). IR spectrum (KBr): 3 470, 3 430, sh 3 365, 3 265, and sh 3 200 cm^{-1} (OH, NH), 1 747, 1 681, and 1 663 cm^{-1} (C=O),

1 604 cm^{-1} (C=N). For $C_8H_{11}N_3O_6$ (245·2) calculated: 39·19% C, 4·52% H, 17·14% N; found: 39·10% C, 4·41% H, 16·93% N.

4-Phenyl-6-methyl-1,2,4-triazin-3,5(2*H*,4*H*)-dione (*X*)

A solution of *VII* (47 mg; 0·2 mmol) in 0·1M methanolic solution of sodium methoxide (3 ml) was allowed to stand for 10 min at room temperature and then neutralized with Dowex 50 (H^+). The resin was filtered off and washed with methanol (5 ml). The combined filtrates were evaporated under diminished pressure. Crystallization of the residue from methanol afforded 26 mg (64%) of *X*, m.p. 245·5–246·5°C (reported[11], m.p. 242·5°C). Crystallization of the mother liquors residue from methanol yielded additional 11 mg (27%) of the same compound. UV spectrum – 0·1M-HCl: λ_{max} 261 nm (log ε 3·76), λ_{min} 229 nm (log ε 3·45); H_2O: λ_{max} 262 nm (log ε 3·79), λ_{min} 230 nm (log ε 3·53); 0·1M-NaOH: λ_{max} 260 nm (log ε 4·13); reported[10], λ_{max} 264 nm, ε 6 360 in ethanol. IR spectrum (KBr): 3 200 cm^{-1} (NH), 1 729 and 1 668 cm^{-1} (C=O), 1 593, sh 1 506, 1 495, and 1 438 cm^{-1} (ring), 1 380 cm^{-1} (CH_3). For $C_{10}H_9N_3O_2$ (203·2) calculated: 59·11% C, 4·46% H, 20·68% N; found: 59·05% C, 4·50% H, 20·81% N.

6-Methyl-4-(β-D-ribofuranosyl)-1,2,4-triazin-3,5(2*H*,4*H*)-dione (*XI*)

A solution of *VIII* (151 mg; 0·25 mmol) in 0·1M methanolic solution of sodium methoxide (4 ml) was allowed to stand for 2 h at room temperature, neutralized with Dowex 50 (H^+), and evaporated under diminished pressure. The residue was chromatographed on a silica gel column (20 g) in the system ethyl acetate–acetone–ethanol–water (40 : 2 : 1 : 1). Compound *XI* (40 mg; 62%) was obtained in the form of a solid foam. The analytical sample was crystallized from the system 2-propanol–ethyl acetate (1 : 1). M.p. 161–164°C; reported[2], 164–165°C. UV spectrum – 0·1M-HCl: λ_{max} 266 nm (log ε 3·68), λ_{min} 228 nm (log ε 3·36); H_2O: λ_{max} 265 nm (log ε 3·72), λ_{min} 231 nm (log ε 3·48); 0·1M-NaOH: λ_{max} 249 nm (log ε 4·08). IR spectrum (KBr): 1 731 and 1 675 cm^{-1} (C=O), 1 380 cm^{-1} (CH_3). For $C_9H_{13}N_6O_6$ (259·2) calculated: 41·70% C, 5·05% H, 16·21% N; found: 41·62% C, 5·27% H, 15·88% N.

The author wishes to thank Mrs J. Hlaváčková for an excellent technical assistance, Dr P. Fiedler for measurement and interpretation of IR spectra, Analytical Department (Dr J. Horáček, Head) of this Institute for elemental analyses, and Mrs Z. Ledvinová for measurement of optical rotations.

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Translated by Z. Točík.