PREPARATION OF 6-AZAURIDINE AND ITS TRIACYL DERIVATIVES*

H. HŘEBABECKÝ and J. BERÁNEK

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

Received November 8th, 1974

Ribosylation of methyl glyoxylate semicarbazone (*I*) silylated derivative with tri-O-benzoyl-D-ribofuranosyl bromide affords the protected 2-ribofuranosyl derivative *IIIa*. Cyclisation of compound *IIIa* with methanolic sodium methoxide at room temperature yields 84% of 6-azauridine (*VIa*) while 6-azauridine tribenzoate *VIb* is obtained in 79% yield by cyclisation of compound *IIIa* with acetic anhydride and sodium acetate at 80°C. Ribosylation of compound *II* with tri-O-acetyl-D-ribofuranosyl chloride and cyclisation affords 6-azauridine triacetate *VIc* in a low yield only (9·5%). A mixture of the amide *V* (38%) and the nucleoside *VIa* (35%) is obtained from compound *IIIa* by the action of methanolic ammonia.

The chemistry of 6-azauridine has been paid attention in this Laboratory over a considerably long period of time. The investigations include isolation of pure 6-azauridine from the fermentation process[1], preparation of nucleotides[2,3] and their use in biochemical assays on the inhibitory effects[4], production of medicinal application forms, *e.g.*, the tri-O-acetyl derivative[5,6], and clinical assays[7]. Furthermore, some 6-azauridine derivatives and analogues modified in the sugar[8–10] or aglycon[11–13] moiety were prepared. The 5-alkyl derivatives[14] of the aglycon component of 6-azauridine and 6-azacytidine have also been examined. The above experiments in the 6-azauridine series do not include the synthesis of the parent 6-azauridine. In this connection and in connection with the earlier preparation of 6-azauridine by ribosylation of 6-azauracil[15,16] it appeared of interest to attempt the ribosylation of glyoxylic acid semicarbazone and the subsequent cyclisation to 6-azauridine. Alkylation of methyl glyoxylate semicarbazone and cyclisation of the resulting product to 1-substituted 6-azauracils were examined as the model reactions. Systematic investigations on alkylations[17] and ribosylations[18] of methyl glyoxylate semicarbazone salts as well as cyclisation of thus-obtained derivatives have been reported in earlier papers[17,18] along with a comparison of the effect of various substituents on the rate and readiness of the cyclisation reaction.

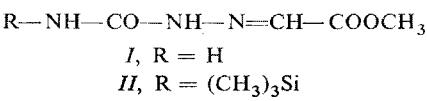
For the ribosylation of methyl glyoxylate semicarbazone at position 2, the silylation process appeared as the most promising. This procedure has been successfully used

* Part III in the series Analogues of Nucleosides; Part II: This Journal *40*, 2378 (1975).

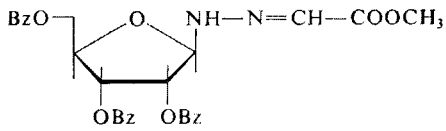
in the direct preparation of tri-O-acetyl-6-azauridine by ribosylation of 6-azauracil with tri-O-acetylribofuranosyl chloride[16]. Since tri-O-acetyl-6-azauridine[6] is known as a suitable peroral medicinal form of 6-azauridine[7] while the free 6-azauridine exhibits serious side effects when administered perorally[19], it was desirable to perform the cyclisation of the tri-O-acetylribofuranosyl derivative *IIIb* to tri-O-acetyl-6-azauridine (*VIc*) without removal of the protecting acetyl groups.

Methyl glyoxylate semicarbazone (*I*) was silylated by heating in hexamethyldisilazane under catalysis of ammonium sulfate[20]. The unreacted hexamethyldisilazane was evaporated and the residual silylation product directly condensed with the corresponding halogenose. The silylation product is probably a disilyl derivative which, however, was not obtained in pure form. On the other hand, its partial methanolysis afforded a pure monosilyl derivative, namely, 4-trimethylsilylsemicarbazone of methyl glyoxylate (*II*), the structure of which was inferred from mass and infrared spectra. The infrared spectrum of compound *II* resembles those of glyoxylic acid 4-ribofuranosylsemicarbazone[18] and glyoxylic acid 4-phenylsemicarbazone[17]. Owing to the trimethylsilyl group as substituent, the wavenumber of the N^4H bond stretching vibration is shifted with respect to 4-ribofuranosylsemicarbazones to lower values than in the case of the 4-phenylsemicarbazone.

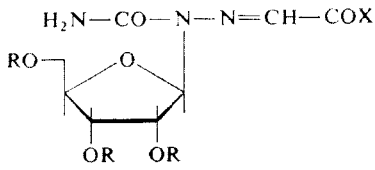
Condensation of the above crude silylated semicarbazone with 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide afforded the protected 2-ribofuranosylsemicarbazone *IIIa*. By the action of sodium hydride in dimethylformamide, the ribosylsemicarbazone *IIIa* is degraded to the protected ribosylhydrazone *IV* in analogy to the degradation



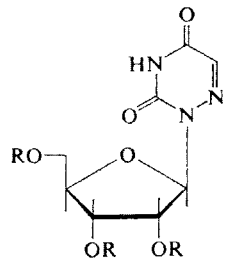
I, R = H
II, R = $(CH_3)_3Si$



IV



IIIa, R = Bz, X = OCH_3
IIIb, R = Ac, X = OCH_3
V, R = H, X = NH_2



VIa, R = H
VIb, R = Bz
VIc, R = Ac

of methyl glyoxylate 2-alkylsemicarbazones[17]. The degradation is not accompanied by removal of benzoyl groups.

The ribosylsemicarbazone *IIIa* may be cyclised to 6-azauridine by the action of methanolic sodium methoxide, methanolic ammonia or acetic anhydride in the presence of sodium acetate. The cyclisation proceeds under considerably milder conditions than cyclisation of the semicarbazone *I* to 6-azauracil or of 2-alkylsemicarbazones to 1-alkyl-6-azauracils[17]. By the action of methanolic sodium methoxide, compound *IIIa* is unambiguously cyclised to the unsubstituted 6-azauridine in 84% yield even at room temperature. When compound *IIIa* is heated in acetic anhydride in the presence of sodium acetate, 2,3,5-tri-O-benzoyl-6-azauridine is formed in 79% yield. In contrast to the analogous cyclisation of 2-alkylsemicarbazones, the reaction mixture did not contain any appreciable amounts of transacylation products or degradation products of the semicarbazone portion of the molecule. By the action of methanolic ammonia, the semicarbazone *IIIa* is converted to 6-azauridine (*VIa*) even at room temperature. The cyclisation and the simultaneous debenzoylation are accompanied by formation of a by-product, the amide *V*. This amide was obtained by work-up of the reaction mixture and crystallisation from ethanol; the mother liquors contained as the main component the free 6-azauridine (*VIa*) which was isolated in the form of tri-O-acetyl-6-azauridine (*VIc*) in 35% yield. At room temperature, the treatment of the amide *V* with methanolic ammonia does not afford 6-azauridine; the cyclisation requires elevated temperatures (4 h at 100°C in an autoclave).

Condensation of the silylated methyl glyoxylate semicarbazone with 2,3,5-tri-O-acetyl-D-ribofuranosyl chloride affords a much lower yield than with the above benzoylated ribofuranosyl bromide. The attempted isolation of the pure acetylated ribofuranosylsemicarbazone *IIIb* by chromatography on silica gel is accompanied by a considerable loss of material. The purification was therefore omitted and the cyclisation performed with the crude condensation product. When heated in acetic anhydride in the presence of sodium acetate, the acetylated condensation product *IIIb* afforded 2',3',5'-tri-O-acetyl-6-azauridine (*VIc*) in 9·5% yield.

TABLE I
Thin-Layer Chromatography

Compound	S_1	Compound	S_2
IIIa	0·37	*I*	0·58
IV	0·65	*V*	0·15
VIb	0·53	*VIa*	0·45
VIc	0·25	6-azauracil	0·79

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block). Analytical samples were dried at 30°C/0·01 Torr for 8 h. Thin-layer chromatography (Table I) was performed on ready-for-use silica gel foils Silufol UV_{254} (Kavalier Glassworks, Votice, Czechoslovakia) in solvent systems S_1, benzene–acetone (4 : 1), and S_2, ethyl acetate–acetone–ethanol–water (12 : 2 : 1 : 1). UV spectra, Optica Milano CF 4 apparatus. IR spectra, Zeiss Model UR 10 apparatus. CD spectra, Roussel-Jouan Dichrograph II Model CD 185 spectropolarimeter. NMR spectra (100 MHz), Varian HA 100 apparatus. Mass spectra, AEI MS 902 apparatus.

Methyl Glyoxylate 4-Trimethylsilylsemicarbazone (*II*)

A mixture of the semicarbazone *I* (725 mg; 5 mmol), hexamethyldisilazane (15 ml), and ammonium sulfate (10 mg) was heated at 150°C (bath temperature) until the semicarbazone dissolved (6 h) and then for 2 h more. Hexamethyldisilazane was evaporated under diminished pressure, the residue coevaporated with toluene (15 ml), then dissolved in toluene (5 ml), the solution treated with methanol (0·2 ml), and kept at room temperature for 30 min to deposit crystals which were collected with suction and washed with light petroleum (2 ml). The solid (740 mg) was sublimed at 120°C (bath temperature) and 20 Torr to afford 700 mg (64·5%) of compound *II*. UV spectrum (cyclohexane): λ_{max} 267 nm (log ε 3·97). IR spectrum (tetrachloromethane): 851 cm^{-1}, 1253 cm^{-1} ($Si(CH_3)_3$), 1516 cm^{-1} (amide II), 1591 cm^{-1} (C=N), 3348 cm^{-1} (N^2H), 3386 cm^{-1} (N^4H). Mass spectrum: M^+ 217, *m/e* 202 $(M - 15)^+$, 158 $(M - 59)^+$, 73 $(Si(CH_3)_3)^+$.

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

A solution of 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide (2 mmol) and the completely silylated methyl glyoxylate semicarbazone (intermediate in the preparation of compound *II*) (2·5 mmol) in acetonitrile (8 ml) was treated under stirring with mercuric bromide (280 mg). The mixture was stirred until the bromide dissolved, kept at room temperature for 20 h under exclusion of atmospheric moisture, and evaporated under diminished pressure. The residue was dissolved in chloroform (50 ml), the solution washed with three 10 ml portions of 10% aqueous potassium iodide and two 10 ml portions of water, dried over anhydrous magnesium sulfate, and evaporated under diminished pressure. The residue (1·5 g) was chromatographed on a column of silica gel (particle size, 30–60 μ; 75 g) in the solvent system benzene–acetone (4 : 1) to afford 366 mg (31%) of a chromatographically homogeneous substance in the form of a solid foam. UV spectrum (ethanol): λ_{max} 232 and 266 nm (log ε 4·52 and 4·07), λ_{min} 256 nm (log ε 4·05). IR spectrum (chloroform): 1556 cm^{-1} (amide II), 1603 cm^{-1} (C=N + ring), 1726 cm^{-1} (CO), 3416 cm^{-1}, 3535 cm^{-1} (NH_2). CD spectrum (ethanol): 198·5 nm (−15450), 234·5 nm (−18310), 281 nm (+17590). NMR spectrum (deuteriochloroform; tetramethylsilane as internal standard; chemical shifts in p.p.m.): 3·70 (s, 3 H, $-COOCH_3$), 4·50–4·90 (m, 3 H, $H_{4'}$, 2 $H_{5'}$), 5·90–6·40 (m, 6 H, $H_{1'}$, $H_{2'}$, $H_{3'}$, $-NH_2$, =CH–), 7·20–7·65, 7·90–8·20 (m, 15 H, arom. protons). For $C_{30}H_{27}N_3O_{10}$ (589·5) calculated: 61·12% C, 4·62% H, 7·13% N; found: 61·02% C, 4·63% H, 7·20% N.

Methyl Glyoxylate 2-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)hydrazone (*IV*)

To a solution of the ribosylsemicarbazone *IIIa* (589 mg; 1 mmol) in dimethylformamide (2 ml) there was added with stirring sodium hydride (30 mg) and the stirring continued for 15 min at room temperature. The mixture was then neutralised with 1M methanolic acetic acid (1·5 ml) and evaporated under diminished pressure. The residue was coevaporated with toluene (4 ml)

and chromatographed on a column of silica gel (particle size, 30—60 μ; 40 g) in the solvent system benzene–acetone (5 : 1). The main absorbing fraction was evaporated to yield 425 mg of a residue which was crystallised from cyclohexane–benzene. Yield, 300 mg of compound *IV*, m.p. 152 to 154°C. Work-up of mother liquors afforded additional crop (105 mg) of the same substance. Total yield, 74%. Optical rotation: $[\alpha]_D^{25}$ −90·87° (*c* 0·46; ethyl acetate). UV spectrum (ethanol): λ_{max} 233 and 272 nm (log ε 4·24 and 4·13), λ_{min} 252 nm (log ε 4·00). IR spectrum (chloroform): 1 587 cm^{-1} (C=N), sh 1 711 cm^{-1} (CO ester), 1 728 cm^{-1} (CO benzoate). For $C_{29}H_{26}N_2O_9$ (546·5) calculated: 63·73% C, 4·80% H, 5·13% N; found: 63·44% C, 4·99% H, 5·22% N.

Glyoxylic Amide 2-β-D-Ribofuranosylsemicarbazone (*V*) and 2′,3′5′-Tri-O-acetyl-6-azauridine (*VIc*)

A solution of the ribosylsemicarbazone *IIIa* (295 mg; 0·5 mmol) in 18% methanolic ammonia (10 ml) was kept at room temperature for 3 days and evaporated under diminished pressure. The residue was dissolved in water (15 ml), the aqueous solution washed with three 5 ml portions of ether, and evaporated under diminished pressure. The residue was crystallised from aqueous ethanol to afford 50 mg (38%) of compound *V*, m.p. 227°C (decomp.). UV spectrum (water): shoulder 241 nm (log ε 3·84), λ_{max} 260 nm (log ε 3·87). IR spectrum (KBr): 1 579 cm^{-1} (C=N), 1 715 cm^{-1} (amide I semicarbazone), 1 675 cm^{-1} (amide I), 1 595 cm^{-1}, 1 633 cm^{-1} (amide II). CD spectrum (water): 198·5 nm ([θ] -15450), 234·5 nm (−18310), 281 nm (+17590). For C_8H_{14} . . N_4O_4 (262·2) calculated: 36·64% C, 5·38% H, 21·37% N; found: 36·48% C, 5·32% H, 21·12% N.

The mother liquors were evaporated, the residue dissolved in a mixture of pyridine (1 ml) and acetic anhydride (0·5 ml), and the whole kept at room temperature for 24 h. Ethanol (0·5 ml) was then added, the solution kept at room temperature for 15 min and evaporated under diminished pressure. The residue was coevaporated with three 3 ml portions of toluene and chromatographed on a column of silica gel (particle size, 30—60 μ; 10 g) in the solvent system benzene–acetone (3 : 2). Crystallisation from di-n-propyl ether yielded 64 mg (35%) of compound *VIc*, m.p. 100—102°C, undepressed on admixture with the authentic 1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-6-azauracil[6]. IR and UV spectra of compound *VIc* were identical with those of the authentic specimen.

6-Azauridine (*VIa*)

A solution of the ribosylsemicarbazone *IIIa* (443 mg; 0·75 mmol) in 0·1M methanolic sodium methoxide (20 ml) was kept at room temperature for 1 h and neutralised with Dowex 50 (H^+) ion exchange resin previously washed with methanol. The resin was filtered off and washed with three 20 ml portions of methanol. The filtrate and washings were combined and evaporated under diminished pressure. The residue was dissolved in water (20 ml), the aqueous solution washed with three 5 ml portions of ether, and evaporated under diminished pressure. Crystallisation of the residue from a mixture of 2-propanol (2·5 ml) and methanol (1·75 ml) yielded 130 mg of compound *VIa*, m.p. 157—158·5°C, undepressed on admixture with authentic 6-azauridine. The mother liquors were evaporated and the residue crystallised as above to afford an additional crop (25 mg) of the same product. Total yield, 84% of compound *VIa*. IR and UV spectra were identical with those of authentic 6-azauridine.

1-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-6-azauracil (*VIb*)

To a solution of the ribosylsemicarbazone *IIIa* (59 mg; 0·1 mmol) in acetic anhydride (1 ml) there was added anhydrous sodium acetate (7 mg), the mixture heated at 80°C for 20 h, and coeva-

porated with toluene to remove acetic anhydride. The residue was crystallised from 2-propanol (2·5 ml) to yield 40 mg of compound *VIb*, m.p. 190·5—192·5°C, undepressed on admixture with an authentic specimen[21]. IR spectra of the two substances were identical. Work-up of mother liquors yielded additional 4 mg of the same product. Total yield, 79% of compound *VIb*.

1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-6-azauracil (*VIc*)

Acetyl chloride (0·6 ml) was added to a solution of 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (637 mg; 2 mmol) in benzene (20 ml) and the whole saturated for 1 h with gaseous hydrogen chloride under cooling with ice. The resulting solution was kept at room temperature for 12 h and evaporated under diminished pressure. The residue was coevaporated with three 5 ml portions of toluene and finally dissolved in acetonitrile (10 ml). The solution was added to 2·5 mmol of the completely silylated methyl glyoxylate semicarbazone (see preparation of compound *IIIa*). When the silyl compound dissolved, mercuric bromide (140 mg) was added. The resulting solution was kept at room temperature for 6 h, the insoluble precipitate filtered off, and washed with two 4 ml portions of acetonitrile. The filtrate and washings were combined and evaporated under diminished pressure. The residue was dissolved in chloroform (25 ml), the solution washed with three 5 ml portions of 10% aqueous potassium iodide and two 5 ml protions of water, dried over anhydrous magnesium sulfate, and evaporated under diminished pressure. The residue was dissolved in acetic anhydride (5 ml), the solution treated with fused sodium acetate (20 mg), and the whole heated at 100°C for 7 h. Acetic anhydride was removed by coevaporation with toluene under diminished pressure and the residue was chromatographed on a thin layer (40 × × 18 × 0·1 cm) of loose silica gel in the solvent system benzene–acetone (3 : 1). The band travelling as 2′,3′,5′-tri-O-acetyl-6-azauridine (*VIc*) was eluted with acetone, the eluate evaporated, and the residue crystallised from 2-propanol to yield 80 mg (9·5%) of a substance, m.p. 100—102°C, undepressed on admixture with authentic[6] *VIc*. IR spectra of the two substances were identical.

The authors wish to thank Dr J. Gut for valuable discussions, Dr M. Masojídková for measurement and interpretation of NMR spectra, and Dr I. Frič for CD spectra. Elemental analyses were kindly perfomed by Mr V. Štěrba. The technical assistance of Mrs J. Hlaváčková is gratefully acknowledged.

REFERENCES

1. Beránek J., Smrt J., Šorm F.: Czechoslov. Pat. 96 759 (1960).
2. Beránek J., Smrt J.: This Journal *25*, 2029 (1960).
3. Smrt J., Beránek J., Šorm F.: This Journal *25*, 2459 (1960).
4. Škoda J.: Progr. Nucleic Acids Res. *2*, 197 (1963).
5. Beránek J., Šorm F.: Czechoslov. Pat. 111 202 (1964).
6. Beránek J., Piťha J.: This Journal *29*, 625 (1964).
7. Grafnetterová J., Beránek J., König J., Šmahel O., Šorm F.: Neoplasma *3*, 241 (1966).
8. Beránek J., Šorm F.: This Journal *33*, 901 (1968).
9. Beránek J.: This Journal *34*, 618 (1969).
10. Farkaš J., Beránek J., Šorm F.: This Journal *31*, 4002 (1966).
11. Wieczorkowski J., Šorm F., Beránek J.: This Journal *33*, 924 (1968).
12. Beránek J., Šorm F.: This Journal *33*, 913 (1968).
13. Beránek J., Šorm F.: This Journal *28*, 546 (1963).
14. Beránek J., Gut J.: This Journal *34*, 2306 (1969).
15. Prystaš M., Šorm F.: This Journal *27*, 1578 (1962).

16. Pískala A., Šorm F.: Czechoslov. Appl. PV 4912 (1973).
17. Hřebabecký H., Beránek J.: This Journal *40*, 2364 (1975).
18. Hřebabecký H., Beránek J.: This Journal *40*, 2378 (1975).
19. Welch A. D., Handschumacher R. E., Finch S. C., Jaffe J. J., Cardoso S. S., Calabresi P.: Cancer Chemotherapy Rep. *9*, 39 (1960).
20. Winkley M. V., Robins R. K.: J. Org. Chem. *33*, 2822 (1968).
21. Černěckij V., Chládek S., Šorm F., Smrt J.: This Journal *27*, 87 (1962).

Translated by J. Pliml.