SYNTHESIS OF 2-AMINO-5-β-D-RIBOFURANOSYL--1,3,4-THIADIAZOLE AND 2-AMINO-β-D-RIBOFURANOSYL--1,3,4-OXADIAZOLE

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Received September 5th, 1985

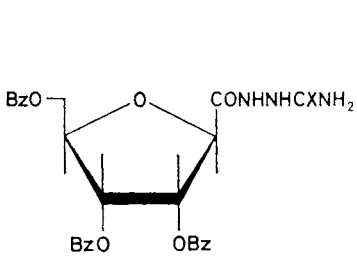
Benzoylated 2-amino-5-ribosylthiadiazole *III* and 2-amino-5-oxadiazole *IV* were synthesized by cyclization of allonoylthiosemicarbazide *I* and allonoylsemicarbazide *II* with phosphorus pentoxide in nitromethane. The oxadiazole *IV* was alternatively prepared by heating compound *I* with lead(II)oxide in acetonitrile. Free ribosylthiadiazole *V* and ribosyloxadiazole *VI* were obtained by methanolysis of *III* and *IV*.

2-Amino-1,3,4-thiadiazole and some of its derivatives are known to exhibit antiviral activity against plant viruses[1], cancerostatic or teratogenic activity[2]. Aminothiadiazole has been found[3] to inhibit inosine monophosphate dehydrogenase (IMP : NAD^+ oxidoreductase, EC 1.2.1.14), the active inhibitor being either 5'-ribonucleotide of this compound or a nicotinamide adenine dinucleotide analogue in which nicotinamide is replaced by aminothiadiazole[4]. However, the location of the ribose–aminothiadiazole bond has not been hitherto determined. There are several other biologically active nucleosides with five-membered heterocyclic bases, such as pyrazofurin[5], showdomycin[6], and thiazofurin[7] whose monophosphates are also potent inhibitors of inosine monophosphate dehydrogenase. These facts gave the impetus to the synthesis of 2-amino-5-β-D-ribofuranosyl-1,3,4-thiadiazole (*V*) and its oxygen analogue oxadiazole *VI*.

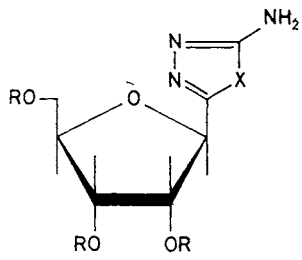
Literature[8] reports the preparation of 2-amino-5-(5-O-acetyl-2,3-O-isopropylidene--β-DL-ribofuranosyl)-1,3,4-oxadiazole by oxidative cyclization of DL-3,4-O-isopropylidene-2,5-anhydroallose semicarbazone. The best approach to the ribosylthiadiazole *V* and the ribosyloxadiazole *VI* seemed to be the reaction scheme starting from 3,4,6-tri-O-benzoyl-2,5-anhydro-D-allonoylthiosemicarbazide (*I*) and the allonoylsemicarbazide *II*, respectively.

Depending on reaction conditions, acylthiosemicarbazides cyclize to various products. Oxidative cyclization with iodine[9,10], mercuric oxide[11], lead(II) oxide[12], or cupric sulfate[13] leads to 2-amino-1,3,4-oxadiazoles. Base-catalyzed cyclization[9–11,14] affords 1,2,4-triazole-3(2*H*)-thiones whereas cyclization in acidic media[9–11,14,15] gives 2-amino-1,3,4-thiadiazoles.

Allonoylthiosemicarbazide *I* and allonoylsemicarbazide *II* were prepared by reaction of 3,4,6-tri-O-benzoyl-2,5-anhydro-D-allonoyl chloride with thiosemicarbazide and semicarbazide, respectively, in acetonitrile. The required 3,4,6-tri-O-benzoyl--2,5-anhydro-D-allonic acid[16] was obtained by hydrolysis of 2,3,5-tri-O-benzoyl--β-D-ribofuranosyl cyanide[16,17]. The acid-catalyzed cyclization of the thiosemicarbazide *I* and the semicarbazide *II* was performed by heating in nitromethane with phosphorus pentoxide. Compound *I* afforded the tri-O-benzoylribofuranosylthiadiazole *III* in 81% yield, and compound *II* gave the tri-O-benzoylribofuranosyloxadiazole *IV* in 66% yield. The oxadiazole *IV* was obtained in 55% yield also by heating the thiosemicarbazide *I* with lead(II) oxide in acetonitrile. Methanolysis of the benzoates *III* and *IV* with methanolic ammonia afforded the respective ribosyl derivatives *V* and *VI*.



I, X = S
II, X = O



III, R = Bz; X = S
IV, R = Bz; X = O
V, R = H; X = S
VI, R = H; X = O

The ^{1}H NMR spectra of the benzoylated thiadiazole *III* ($H_{1'}$ at δ 5·52 ppm; $J_{1',2'}$ = 5·5 Hz), benzoylated oxadiazole *IV* ($H_{1'}$ at δ 5·41 ppm; $J_{1',2'}$ = 5 Hz), free thiadiazole *V* ($H_{1'}$ at δ 4·77 ppm; $J_{1',2'}$ = 5·5 Hz) and oxadiazole *VI* ($H_{1'}$ at δ 4·65 ppm; $J_{1',2'}$ = 6 Hz) are in accord with those of C-nucleosides with five--membered heterocyclic bases and β-anomeric configuration[18,19].

Ribosylthiadiazole *V* exhibited 39% inhibition of the growth of *Escherichia coli B* in a synthetic medium in concentration 5 . 10^{-4} mol l^{-1}.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block). Ultraviolet spectra were recorded on a Specord apparatus, IR spectra on a UR-20 (Carl Zeiss, Jena) instrument. ^{1}H NMR spectra were recorded on a Tesla BS 467 60 MHz spectrometer, using tetramethylsilane as internal standard; chemical shifts (δ values) are expressed in ppm and coupling constants in Hz. Column chromatography was performed on Pitra silica gel (particle size 30—60 μm; produced by Service Laboratories of this Institute).

3,4,6-Tri-O-benzoyl-2,5-anhydro-D-allonoylthiosemicarbazide (*I*)

A mixture of 3,4,6-tri-O-benzoyl-2,5-anhydro-D-allonic acid[16] (1·96 g; 4 mmol), benzene (5 ml), thionyl chloride (5 ml), and dimethylformamide (0·1 ml) was heated to 100°C (bath) for 3 h. After evaporation *in vacuo*, the residue was coevaporated with benzene (3 × 10 ml) and dissolved in acetonitrile (15 ml). After addition of thiosemicarbazide (600 mg) to the stirred solution, triethylamine (0·37 ml) was added dropwise over 10 min. The mixture was stirred for 3 h at room temperature, diluted with ethyl acetate (250 ml), washed with water (3 × 50 ml), saturated sodium chloride solution (50 ml), dried over magnesium sulfate and taken down *in vacuo*. Chromatography of the residue on a column of silica gel (200 g) in toluene–ethyl acetate (2 : 3) afforded 1·54 g (68%) of *I* as a solid foam. IR spectrum (chloroform), cm^{-1}: 3 511 and sh 3 391 (NH_2), sh 3 274 and 3 366 (NH), 1 729 and 1 713 (C=O benzoate), sh 1 680 (amide *I*), 1 604, sh 1 587, 1 496 and 1 454 (phenyl ring), sh 1 522 and sh 1 542 (amide *II*), 1 273 (C—O benzoate). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 4·62 (broad s, 3 H, $H_{4'}$, $H_{5'}$, $H_{5''}$), 4·82 (d, 1 H, $H_{1'}$, $J_{1',2'} = 3$), 5·67–6·07 (m, 2 H, $H_{2'}$, $H_{3'}$), 7·18–8·17 (m, 17 H, NH_2, H benzoate), 9·33 (broad s, 1 H, NH), 10·25 (broad s, 1 H, NH). For $C_{28}H_{25}N_3O_8S$ (563·6) calculated: 59·67% C, 4·47% H, 7·46% N, 5·69% S; found: 59·40% C, 4·48% H, 7·35% N, 5·41% S.

3,4,6-Tri-O-benzoyl-2,5-anhydro-D-allonoylsemicarbazide (*II*)

A mixture of 3,4,6-tri-O-benzoyl-2,5-anhydro-D-allonic acid[16] (981 mg; 2 mmol), benzene (2·5 ml), thionyl chloride (2·5 ml), and dimethylformamide (50 μl) was heated to 100°C (bath) for 3 h. After cooling, the solution was taken down *in vacuo*, the residue was coevaporated with benzene (3 × 5 ml) and dissolved in acetonitrile (5 ml). Semicarbazide (380 mg) was added, the mixture was stirred for 4·5 h at room temperature, diluted with ethyl acetate (150 ml), washed with water (3 × 20 ml) and saturated sodium chloride solution (20 ml), dried over magnesium sulfate and the solvents were evaporated *in vacuo*. Crystallization of the residue from ethanol afforded 511 mg (46·5%) of allonoylsemicarbazide *II*, m.p. 155–158°C. Column chromatography of the mother liquors on silica gel (50 g) in ethyl acetate–2-propanol (96 : 4) and subsequent crystallization from ethanol afforded further portion (150 mg; 14%) of *II*. IR spectrum (chloroform) *c* 0·003 mol . $.l^{-1}$, cm^{-1}: 3 403 and 3 523 (NH_2), 3 307 (NH); *c* 2%: 1 728 and sh 1 712 (C=O benzoate), sh 1 692 and sh 1 681 (amide *I*), 1 602, 1 587 and sh 1 495 (phenyl ring), sh 1 525 and 1 510 (amide *II*), 1 272 (C—O benzoate). ^{1}H NMR spectrum (deuteriochloroform + hexadeuteriodimethyl sulfoxide): 4·70 (broad s, 3 H, $H_{4'}$, $H_{5'}$, $H_{5''}$), 4·85 (d, 1 H, $H_{1'}$, $J_{1',2'} = 3$), 5·58 (broad s, 2 H, NH_2), 5·62–6·12 (m, 2 H, $H_{2'}$, $H_{3'}$), 7·13–7·67, 7·73–8·17 (m, 15 H, H benzoate), 9·31 (broad s, 2 H, N^1H, N^2H). For $C_{28}H_{25}N_3O_9$ (547·5) calculated: 61·42% C, 4·60% H, 7·67% N; found: 61·42% C, 4·61% H, 7·65% N.

2-Amino-5-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,3,4-thiadiazole (*III*)

Phosphorus pentoxide (1·7 g) was added at 100°C to a stirred solution of allonoylthiosemicarbazide *I* (1·69 g; 3 mmol) in nitromethane (30 ml). The mixture was stirred at 100°C for 35 min, cooled, diluted with ethyl acetate (300 ml), washed with saturated solution of sodium hydrogen carbonate (2 × 80 ml), water (80 ml), saturated solution of sodium chloride (50 ml) and dried over magnesium sulfate. The solvents were removed *in vacuo* and the residue was chromatographed on a column of silica gel (150 g) in ethyl acetate–toluene (3 : 2) to give 1·47 g (81%) of thiadiazole *III* as a solid foam. IR spectrum (chloroform) *c* 0·003 mol l^{-1}, cm^{-1}: 3 499 and 3 396 (NH_2); *c* 2%: 1 729 and sh 1 712 (C=O benzoate), 1 603, 1 586 and 1 499 (phenyl ring), sh 1 522 and sh 1 509 (thiadiazole ring), 1 271 (C—O benzoate). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 4·47–4·88 (m, 3 H, $H_{4'}$, $H_{5'}$, $H_{5''}$), 5·52 (d, 1 H, $H_{1'}$, $J_{1',2'} = 5·5$), 5·67–6·13 (m,

2 H, $H_{2'}$, $H_{3'}$), 7·17—7·72, 7·78—8·17 (m, 17 H, NH_2, H benzoate). For $C_{28}H_{23}N_3O_7S$ (545·5) calculated: 61·64% C, 4·25% H, 7·70% N, 5·88% S; found: 61·66% C, 4·45% H, 7·48% N, 5·65% S.

2-Amino-5-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,3,4-oxadiazole (*IV*)

A) Phosphorus pentoxide (500 mg) was added at 100°C to a stirred solution of allonoylsemicarbazide *II* (547 mg; 1 mmol) in nitromethane (10 ml). The mixture was stirred at 100°C for 45 min, cooled, diluted with ethyl acetate (100 ml), washed with saturated solution of sodium hydrogen carbonate (2 × 30 ml), water (30 ml), saturated solution of sodium chloride (20 ml) and dried over magnesium sulfate. The solvents were evaporated *in vacuo* and the residue was crystallized from ethanol, affording 350 mg (66%) of oxadiazole *IV*, m.p. 218—220°C. IR spectrum (KBr), cm^{-1}: 3 280 and 3 128 (NH_2), 1 725 (C=O benzoate), 1 658 (NH_2), 1 615, 1 604, 1 586, 1 494 and 1 453 (phenyl ring and oxadiazole), 1 272 and 1 283 (C—O benzoate). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 4·47—4·97 (m, 3 H, $H_{4'}$, $H_{5'}$, $H_{5''}$), 5·41 (d, 1 H, $H_{1'}$, $J_{1',2'} = 5$), 5·68—6·18 (m, 2 H, $H_{2'}$, $H_{3'}$), 7·19 (s, 2 H, NH_2), 7·32—7·70, 7·80—8·10 (m, 15 H, H benzoate). For $C_{28}H_{23}N_3O_8$ (529·5) calculated: 63·51% C, 4·38% H, 7·94% N; found: 63·30% C, 4·18% H, 7·80% N.

B) A solution of allonoylthiosemicarbazide *I* (282 mg; 0·5 mmol) in acetonitrile (5 ml) was refluxed for 8 h with lead(II) oxide (500 mg). After cooling, the mixture was diluted with ethyl acetate (50 ml) and filtered through a thin layer of Celite. The solid on the filter was washed with ethyl acetate (20 ml) and the combined filtrates were taken down *in vacuo*. Crystallization of the residue from ethanol afforded 130 mg (49%) of oxadiazole *IV*, m.p. 217—220°C; no depression on admixture with product obtained under *A*. The mother liquors afforded further 16 mg (6%) of the product. The IR spectrum was identical with that of the product prepared under *A*.

2-Amino-5-β-D-ribofuranosyl-1,3,4-thiadiazole (*V*)

A solution of benzoyl derivative *III* (546 mg; 1 mmol) in methanolic ammonia (saturated at 20°C; 15 ml) was set aside for 2 days at 3°C. After evaporation *in vacuo*, the residue was washed with ether (2 × 5 ml) and chromatographed on a column of silica gel (50 g) in ethyl acetate–acetone–ethanol–water (14 : 3 : 4 : 4). Crystallization from 2-propanol–methanol afforded 120 mg (51%) of thiadiazole *V*, m.p. 166—168°C. Mother liquors on crystallization afforded further 50 mg (21%) of the same product. UV spectrum (water): λ_{max} 261 nm (log ε 3·81); IR spectrum (KBr), cm^{-1}: 3 454, 3 394, 3 281 and 3 148 (NH_2, OH), 1 640 (NH_2), 1 523 and sh 1 517 (thiadiazole ring). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 3·28—3·58 (m, 2 H, $H_{5'}$, $H_{5''}$), 3·67—4·15 (m, 3 H, $H_{2'}$, $H_{3'}$, $H_{4'}$), 4·58—5·07 (m, 3 H, 2 OH, $H_{1'}$), 5·20 (d, 1 H, OH, $J = 6$), 7·12 (s, 2 H, NH_2); after exchange with deuterium oxide: 4·77 (d, 1 H, $H_{1'}$, $J_{1',2'} = 5·5$). For $C_7H_{11}N_3O_4S$ (233·2) calculated: 36·04% C, 4·75% H, 18·02% N, 13·75% S; found: 36·02% C, 4·66% H, 18·12% N, 13·67% S.

2-Amino-5-β-D-ribofuranosyl-1,3,4-oxadiazole (*VI*)

Benzoyl derivative *IV* (529 mg; 1 mmol) was methanolyzed in the same manner as described for the benzoyl derivative *III*. Crystallization from 2-propanol–methanol afforded 170 mg (78%) of oxadiazole *VI*, m.p. 161—162·5°C. UV spectrum (water): λ_{max} 225 nm (log ε 3·89); IR spectrum (KBr), cm^{-1}: 3 457, 3 305 and 3 215 (NH_2, OH), 1 669 (NH_2), 1 632, 1 600 and sh 1 593 (oxadiazole ring). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 3·45 (d, 2 H, $H_{5'}$, $H_{5''}$, $J_{5',4'} = J_{5'',4'} = 5$), 3·62—4·32 (m, 3 H, $H_{2'}$, $H_{3'}$, $H_{4'}$), 4·67 (q, 2 H, $H_{1'}$, OH), 5·00 (d, 1 H, OH, $J = 5$), 5·24 (d, 1 H, OH, $J = 6$), 7·04 (s, 2 H, NH_2); after exchange with deuterium oxide:

4·65 (d, 1 H, $H_{1'}$, $J_{1',2'} = 6$). For $C_7H_{11}N_3O_5$ (217·2) calculated: 38·71% C, 5·11% H, 19·35% N; found: 38·78% C, 5·07% H, 19·19% N.

The author is indebted to Mrs J. Hlaváčková for the excellent technical assistance, Dr P. Fiedler for interpretation of the IR *spectra, Mrs J. Jelínková for* ^{1}H NMR *spectral measurements and the staff of the Analytical Laboratory (Dr J. Horáček, Head) of this Institute for elemental analyses and Dr J. Votruba for antibacterial tests.*

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Translated by M. Tichý.