UTILIZATION OF 1,6-ANHYDROHEXOSES FOR THE PREPARATION OF SOME ALIPHATIC ADENOSINE ANALOGS*

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Dedicated to Prof. J. Staněk on the occasion of his 70th birthday.

Reaction of sodium salt of adenine with 1,6:3,4-dianhydro-2-O-p-toluenesulfonyl-β-D-galactopyranose (*I*) afforded 4-(adenin-9-yl)-1,6:2,3-dianhydro-4-deoxy-β-D-mannopyranose (*II*) and 2,4-bis(adenin-9-yl)-1,6-anhydro-2,4-dideoxy-β-D-glucopyranose (*IV*). Compound *II* was converted into 4-(adenin-9-yl)-1,6-anhydro-4-deoxy-β-D-glucopyranose (*VI*). Cleavage of the 1,6-anhydro bond in this compound with hot concentrated hydrochloric acid led to 4-(adenin-9-yl)-4-deoxy-D-glucose (*VIII*) which was reduced with sodium borohydride to give 4-(adenin-9-yl)-4-deoxy-D-glucitol (*IX*). Epoxide *II* was reduced with lithium aluminium hydride and the obtained 4-(adenin-9-yl)-1,6-anhydro-2,4-dideoxy-β-D-*arabino*-hexopyranose (*VII*) on treatment with dilute hydrochloric acid and subsequent reduction with sodium borohydride gave 4-(adenin-9-yl)-2,4-dideoxy-D-*arabino*-hexitol (*XI*).

In connection with investigation of chemically modified nucleosides[1] considerable attention has been paid recently to their acyclic analogs in which the furanose ring of the nucleoside is replaced by a hydroxylated carbon chain.

Synthesis of this type of compounds was prompted by the discovery of antiviral activity of acyclovir, its analogs and chiral 9-(*S*)-(2,3-dihydroxypropyl)adenine ((*S*)-DHPA)) (ref.[2]), and the connection between antiviral activity of (*S*)-DHPA and its inhibition of S-adenosyl-L-homocysteine hydrolase[3,4] (SAH-hydrolase, EC 3.3.1.1).

The SAH-hydrolase inhibition has been studied with many alkyl derivatives of purine bases[5-8]. Most of the active compounds were adenine derivatives in which the aliphatic chain was attached in position 9 and contained a 2,3-vicinal diol grouping with *S*-configuration in position 2 (refs[5,7]). Another group of potent SAH-hydrolase inhibitors comprises hydroxylated 9-(ω-carboxyalkyl)adenines[8]. Relati-

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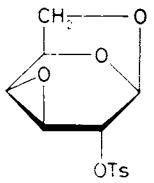
vely little in this respect is known about acyclic adenosine derivatives with a branched side-chain which are more difficult to obtain[9].

The aim of the present study was to elaborate a novel approach to another type of 9-(polyhydroxyalkyl)adenines, represented by the hitherto undescribed 4-(adenin-9-yl)hexitols *IX* and *XI*. Our synthesis utilizes oxirane derivatives of 1,6-anhydrohexoses, easily accessible from 1,6-anhydro-β-D-glucopyranose (levoglucosan). It involves the formation of 1,6-anhydrohexoses bearing adenine moiety bonded by the non-glycosidic bond in position 4, opening the 1,6-anhydride ring and subsequent reduction of the free sugar under formation of the corresponding alditol derivative.

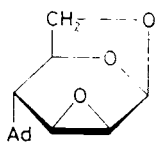
The starting compound, 1,6 : 3,4-dianhydro-2-O-*p*-toluenesulfonyl-β-D-galactopyranose (*I*), was readily obtained from levoglucosan[10]. Levoglucosan is frequently used in the saccharide syntheses[11] and the preparation of hydroxyalkyl derivatives of adenine represents thus another utilization of this compound.

The attachment of adenine moiety to the sugar ring was achieved by reaction of adenine sodium salt (prepared in situ by treatment of the base with sodium hydride in dimethylformamide) with the epoxide *I*. Although the opening of the epoxide ring in compound *I* with nucleophiles is a well-investigated higly regioselective reaction[11] leading to 4-substituted derivatives of D-*gluco*-configuration, the only nitrogen nucleophile used so far in this reaction was ammonia[12]. This reaction proceeds via 4-amino-1,6-anhydro-4-deoxy-2-O-*p*-toluenesulfonyl-β-D-glucopyranose which in the basic medium is converted to 4-amino-1,6 : 2,3-dianhydro-4-deoxy-β-D-mannopyranose, accompanied by 1,6-anhydro-3,4-dideoxy-3,4-epimino-β-D-altropyranose. The cleavage of the oxirane ring in compound *I* by the adenine anion proceeds analogously giving, however, 4-(adenin-9-yl)-1,6:2,3-dianhydro-4-deoxy-β-D-mannopyranose (*II*) as the sole product. The formation of the assumed intermediate, i.e. 4-(adenin-9-yl)-1,6-anhydro-4-deoxy-2-O-*p*-toluenesulfonyl-β-D-glucopyranose (*III*), has not been detected at all. Under the reaction conditions, the intermediate *III* is immediately transformed to the 2,3-oxirane derivative *II* which subsequently reacts with the adenine salt, still present in the reaction mixture, to give 2,4-bis(adenin-9-yl)-1,6-anhydro-2,4-dideoxy-β-D-glucopyranose (*IV*) as the final reaction product. This assumption has been verified by an independent reaction of epoxide *II* with adenine sodium salt under the comparable conditions affording quantitatively the diadenyl derivative *IV*, and also by reaction of the starting epoxide *I* with two equivalents of adenine sodium salt, which gave the derivative *IV* in high yield. In the latter case, we observed the formation of compound *II* which then disappeared from the reaction mixture.

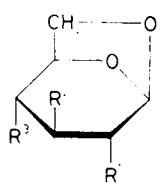
To ensure optimum formation of the required product *II*, it is advisable to avoid an excess of the adenine salt in the reaction with epoxide *I* and to stop the reaction before the appearance of derivative *IV*. The unreacted compound *I* can be readily recovered from the reaction mixture by chromatography.



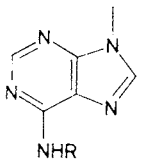
I



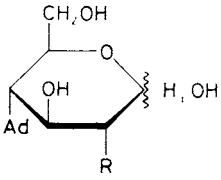
II



III R^1 = OTs, R^2 = OH, R^3 = Ad
IV, R^1 = R^3 = Ad, R^2 = OH
V, R^1 = R^3 = Ad(Ac), R^2 = OAc
VI, R^1 = R^3 = OH, R^2 = Ad
VII, R^1 = H, R^2 = OH, R^3 = Ad



Ad: R = H
Ad(Ac): R = Ac



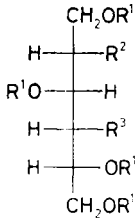
VIII, R = OH
X, R = H

Hydrolysis of the oxirane ring converted the derivative *II* into 4-(adenin-9-yl)-1,6--anhydro-4-deoxy-β-D-glucopyranose (*VI*). This reaction can be catalyzed both by acids and bases; however, the acid hydrolysis (0·5M-H_2SO_4) gave higher yields and the isolation was facile. No cleavage of the 1,6-anhydride bond took place under these conditions. On the other hand, alkaline hydrolysis (1M-NaOH) of compound *II* was accompanied by side-reactions, affording thus lower yields of the required compound *VI*. The unidentified side-products were rather polar, readily soluble in water and probably of oligomeric or polymeric character.

Key reaction in our synthesis of the glucitol derivative *IX* was the cleavage of the 1,6-anhydride bond in compound *VI*. This bond appeared to be unusually stable and application of conventional methods[11], used for conversion of 1,6-anhydrohexoses to hexoses, failed. A difficult cleavage of this bond was reported mainly for 1,6-anhydrohexoses containing an electronegative group such as -OTs, -F, -$NHCOCH_3$, -NH_3^+, etc., on the C-2 carbon atom. In this context, the levoglucosan derivative, substituted with adenin-9-yl residue in position 4, represents a novel, so far not investigated, type of compounds. X-Ray diffraction analysis of the crystalline dihydrate *VI* afforded information about its stereochemistry and parameters of the unit cell[13]. According to these measurements, there is no steric hindrance to approach of the cleavage reagent to the 1,6-anhydride bond oxygen atom. It is thus probable that the difficult cleavage is due to a strong electronegative effect

of the adenine ring. The 1,6-anhydride bond in compound *VI* resisted dilute (0·05 mol. $.l^{-1}$) sulfuric acid (higher concentrations of the acid ($0{\cdot}1-2{\cdot}5$ mol l^{-1}) caused complete degradation), trifluoroacetic acid or dilute hydrochloric acid. Attempted acetolysis with acetic anhydride and trifluoroacetic acid led only to the peracetyl derivative from which deacetylation recovered the starting compound. The same result was obtained in the attempted cleavage with hydrogen bromide in a mixture of acetic acid and acetic anhydride, with bromotrimethylsilane or iodotrimethylsilane (after silylation with hexamethyldisilazane), and with boron tribromide in dichloromethane at −78°C.

The only way which led to the cleavage of the 1,6-anhydro bond in compound *VI* consisted in heating with concentrated hydrochloric acid. The free glucose derivative *VIII* was not stable enough to allow isolation in the pure state; therefore, the reaction mixture after hydrolysis of compound *VI* and neutralization was directly reduced with sodium borohydride to give stable 4-(adenin-9-yl)-4-deoxy-D-glucitol (*IX*).



IX, $R^1 = H$; $R^2 = OH$; $R^3 = Ad$
XI, $R^1 = R^2 = H$; $R^3 = Ad$
XII, $R^1 = Ac$; $R^2 = H$; $R^3 = Ad$
XIII, $R^1 = Ac$; $R^2 = H$; $R^3 = Ad(Ac)$

To obtain the 2-deoxy derivative *XI*, we first prepared 4-(adenin-9-yl)-1,6-anhydro--2,4-dideoxy-β-D-*arabino*-hexopyranose (*VII*) by reduction of 4-(adenin-9-yl)-4-deoxy--1,6:2,3-dianhydro-β-D-mannopyranose (*II*) with lithium aluminium hydride in tetrahydrofuran[14]. The difficult isolation of the product lowered the yields to only about 30%. Owing to the known propensity of adenine to form complexes with metal ions, a substantial part of the product remained in the precipitated hydrated aluminium hydroxides after decomposition of the reaction mixture.

The 1,6-anhydro bond in derivatives of 2-deoxy-1,6-anhydrohexoses is cleaved generally more readily[11] than in compounds having free hydroxyl in position 2. This has been confirmed also in the case of compound *VII* in which the 1,6-anhydro ring was opened already by warming with 0·5M hydrochloric acid. The final product, 4-(adenin-9-yl)-2,4-dideoxy-D-*arabino*-hexitol (*XI*), was obtained in a good yield by reduction with sodium borohydride without isolation of the free deoxyglucose derivative *X*. The structure of the starting compound *I* and all the new compounds

II, *IV*–*VII*, *IX* and *XI*–*XIII* was confirmed by their ^{1}H NMR and ^{13}C NMR spectra (Tables I, II).

Values of the geminal coupling constants $J(6, 6')$ in the spectra of compounds *I*–*VII* (−7 to −8 Hz) prove the presence of the 1,6-anhydro bond. Moreover, the conformational fixation of protons on C-6, which is a part of the five-membered ring, is manifested by characteristic values[11,15,16] of the coupling constants $J(5, 6en)$ (<2 Hz) and $J(5, 6ex)$ (>5 Hz).

The oxirane ring in positions 3 and 4 in the starting compound *I* is confirmed by the upfield position of the H-3 and H-4 proton signals (δ 3·11 and 3·80), and particularly of the C-3 and C-4 carbon atom signals (δ 46·91 and 52·83). The *galacto*--configuration follows from the vicinal coupling constants of protons in position 1–5 (Table I), very similar to those found for 1,6:3,4-dianhydro-β-D-galactopyranose[16].

The position of the 2,3-oxirane ring in compound *II* follows from the upfield shift of the H-2 and H-3 proton signals (δ 3·75 and 3·45) and the C-2 and C-3 carbon signals (δ 53·90 and 48·19). The *manno*-configuration of this compound is proven by coupling constant values that are very close to those for 1,6:2,3-dianhydro-β-D--mannopyranose[16]. The replacement of the 4-hydroxy group with the adenin-9-yl residue results in a marked downfield shift of the H-4 proton signal (1·31 ppm) and smaller downfield shifts of all other signals of protons in the molecule (0·33 to 0·54 ppm) without any apparent decrease with increasing distance from the substitution site. The resulting effect thus seems to be due to combination of the inductive effect and magnetic anisotropy of the heterocyclic moiety. On the other hand, in the ^{13}C NMR spectra this substitution is accompanied with a very marked upfield shift of the C-4 signal (−16·1 ppm) and substantially weaker upfield shifts of the neighbouring (C-3, C-5) carbon signals (−1·2 and −2·5 ppm).

The presence of two adenin-9-yl moieties in compound *IV* has been unequivocally proven by doubled adenin-9-yl proton and carbon signals in the NMR spectra. The spectrum of diadenin-9-yl derivative *IV* exhibited an unusual set of coupling constants: $J(1, 2) \approx 0$, $J(2, 3) = 8{\cdot}0$, $J(3, 4) = 8{\cdot}5$ and $J(4, 5) = 1{\cdot}4$ Hz. Such high values of $J(2, 3)$ and $J(3, 4)$ would be consistent with a reversed (*ido*) configuration at C-2, C-3 and C-4. However, such a structure is excluded both by chemical arguments and the values of the remaining $J(1, 2)$ and $J(4, 5)$ constants (for 1,6--anhydro-β-D-idopyranose reported[15] J (1, 2) = 1·5 and $J(4, 5) = 4{\cdot}1$ Hz). All the observed coupling constants are compatible with the less common boat conformation ($B_{0,3}$) of the pyranose ring of compound *IV*. Obviously the chair form ($^1C_4$) in compound *IV* is destabilized by steric (and possibly also dipolar) interactions between the two 2,4-diaxial adenin-9-yl moieties. The boat conformation $B_{0,3}$ has been proven already earlier in 2,4-diamino-1,6-anhydro-2,4-dideoxy-β-D-glucopyranose, the analogous diazido derivative[17] and 3-amino-1,6-anhydro-3-deoxy-β-D-glucopyranose[18].

TABLE I

Proton NMR parameters of compounds *I*, *II*, *IV*–*VII*, *IX*, *XI*–*XIII* in CD_3SOCD_3

	I[a]	*II*[b]	*IV*[c]	*V*[d]	*VI*[e]	*VII*[f]	*IX*[g]	*XI*[h]	*XII*[i]	*XIII*[j]
					Chemical shifts (δ)					
Carbohydrate										
H-1	5·18	5·98	5·68	6·03	5·61	5·64	3·38	3·42	3·92	3·91
H-1′	—	—	—	—	—	—	3·24	3·42	3·92	3·91
H-2(α)	—	3·75	—	—	—	2·27	3·13	1·27	1·66	1·69
H-2′(β)	4·33	—	4·46	4·85	3·73	1·78	—	0·99	1·24	1·20
H-3	3·11	3·45	4·70	5·84	3·97	3·93	4·39	4·42	5·50	5·53
H-4	3·80	5·07	4·59	5·10	4·78	4·62	4·58	4·42	5·08	5·20
H-5	5·06	4·67	5·01	5·32	4·76	4·64	4·05	4·07	5·62	5·68
H-6(*en*)	3·78	3·97	4·20	4·37	4·47	4·38	3·10	3·12	3·96	4·00
H-6′(*ex*)	3·43	3·77	3·75	3·87	3·91	3·70	3·04	3·06	3·71	3·72
Adenine										
H-2	—	8·26	8·17; 8·18	8·51; 8·59	8·37	8·16	8·08	8·07	8·17	8·67
H-8	—	8·27	8·20; 8·30	8·64; 8·67	8·43	8·19	8·12	8·12	8·33	8·67
NH	—	7·38	7·28	7·30	7·31	7·23	7·15	7·18	7·30	10·72

Coupling constants (Hz)

$J(1, 1')$	—	—	—	—	—	—	10·8	[k]	[k]	[k]
$J(1, 2)$	—	3·2	—	—	—	2·3	4·5	[l]	[k]	[k]
$J(1, 2')$	1·0	—	≈0	≈0	≈1·5	1·4	—	[l]	[k]	[l]
$J(1', 2)$	—	—	—	—	—	—	5·9	[l]	[k]	[l]
$J(1', 2')$	—	—	—	—	—	—	—	[l]	[k]	[l]
$J(2, 2')$	—	—	—	—	—	15·3	—	13·9	14·4	
$J(2, 3)$	—	3·7	—	—	—	5·9	5·5	4·6	4·0	4·0
$J(2', 3)$	0·4	—	8·0	8·0	≈1·5	1·6	—	9·0	8·9	8·9
$J(3, 4)$	4·0	0·6	8·5	8·0	≈1·5	1·5	3·2	[k]	2·3	2·3
$J(4, 5)$	5·0	1·1	1·4	1·3	≈1·5	1·5	8·6	9·1	10·6	10·6
$J(5, 6)$	0·7	1·9	≦0·5	≦0·5	1·0	1·1	4·2	4·2	2·4	2·4
$J(5, 6')$	4·8	6·6	5·0	5·2	5·8	5·9	5·4	5·2	4·0	3·6
$J(6, 6')$	6·5	7·6	7·7	8·0	7·4	7·5	11·2	11·4	12·8	12·8

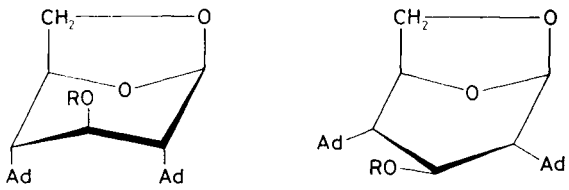
Additional parameters: [a] $J(1, 3) = 1·6$, $J(2, 4) = 0·9$, $J(2, 5) = 0·6$ Hz; $SO_2C_6H_4CH_3$: 7·97 m (2 H), 7·57 m (2 H), 2·50 s (3 H); [b] $J(2, 4) = 0·7$, $J(3, 5) = 1·4$ Hz; [c] OH: 5·68 d ($J = 6·4$ Hz); [d] OAc: 1·65 s; 2 × NAc: 2·26 s, 2·27 s; [e] $J(1, 3) = 1·2$, $J(2, 4) = J(3, 5) = 1·5$ Hz; [f] OH: 5·42 d ($J = 3·6$ Hz); $J(1, 3) = 1·0$, $J(1, 4) = J(2', 5) = 0·8$, $J(2', 4) = 1·5$ Hz; all J-values taken from the spectrum in CD_3OD which gave better resolution; [g] 5 × OH: 4·59 d ($J = 3·2$), 4·55 d ($J = 3·3$), 4·47 t ($J = 5·5$), 4·40 t ($J = 5·5$), 4·39 d ($J = 5·0$ Hz); [h] 4 × OH: 5·21 d ($J = 5·0$), 5·18 d ($J = 4·0$), 4·48 t ($J = 5·5$), 4·33 t ($J = 5·2$ Hz); [i] 4 × OAc: 2·12 s, 2·08 s, 1·95 s, 1·88 s; [j] 4 × OAc: 2·11 s, 2·09 s, 1·95 s, 1·87 s; NAc: 2·28 s; [k] the J-value could not be determined; [l] only a sum of $J(1, 2) + J(1', 2) = 13·8$ Hz and $J(1, 2') + J(1', 2') = 11·8$ Hz is accessible.

TABLE II

Carbon-13 chemical shifts of compounds *I*, *II*, *IV*–*VII*, *IX*, *XI*–*XIII* in CD_3SOCD_3

Carbon	*I*[a]	*II*	*IV*	*V*[b]	*VI*	*VII*	*IX*	*XI*	*XII*[c]	*XIII*[d]
Carbohydrate										
C-1	97·29	97·41	102·38	101·58	102·31	99·64	62·48	57·77	60·32	60·24
C-2	71·26*	53·90	60·96*	58·31*	70·55*	36·18	68·19	37·43	30·53	30·49
C-3	46·91	48·19	67·99	70·60	71·68*	65·09	72·70	64·73	61·63	61·49
C-4	52·83	50·94	62·10*	59·67*	56·40	57·65	57·37	59·18	54·58	55·06
C-5	72·33*	71·71	76·17	75·43	74·33	73·98	70·83	70·49	67·21	67·08
C-6	64·63	66·46	69·29	69·16	65·95	65·59	63·20	63·35	68·08	67·91
Adenine										
C-2	—	152·86	152·72 152·62	152·06** 151·94**	152·50	152·59	152·25	152·41	153·09	152·14*
C-4	—	149·28	149·96 149·84	149·67 149·67	149·20	149·30	150·14	150·29	150·26	149·79
C-5	—	118·39	119·15 118·96	123·23 123·06	118·35	118·35	118·24	118·02	119·83	122·41
C-6	—	156·25	156·18 156·18	152·01** 151·85**	156·18	156·18	156·06	156·08	156·25	152·58*
C-8	—	139·04	139·73 138·93	142·98 142·41	141·18	139·45	141·33	140·82	140·13	143·46

Signals with the same symbols (* or **) may be interchanged. Additional signals: [a] $SO_2C_6H_4CH_3$: 145·92, 130·63(2), 127·96(2), 132·30, 21·30; [b] OAc: 169·10, 20·05; 2 × NAc: 169·01(2), 24·54(2); [c] OAc: 170·34, 170·05, 169·84, 169·58, 20·88(2), 20·76, 20·54; [d] NAc: 169·01, 24·56; OAc: 170·26, 169·92, 169·74, 169·50, 20·82(2), 20·69, 20·47.



$^1C_4$ $B_{0,3}$

Triacetate *V* (obtained by acetylation of compound *IV*) also exists in a boat conformation, as shown by vicinal coupling constants of the pyranose ring protons (cf. data for *IV* and *V* in Table I).

In compound *VI*, formed by hydrolysis of the epoxide *II*, the 1,6-anhydro bond remains intact ($J(6,6') = -7{\cdot}4$ Hz). The configuration on the C-2, C-3 and C-4 carbon atoms follows from the very small vicinal coupling constants of pyranose ring protons ($\sim 1{\cdot}5$ Hz) and from the approximately equal coupling constants across four bonds between protons in positions 1,3, 2,4 and 3,5. These data are characteristic of 1,6-anhydro derivatives of *gluco*-configuration[15] with chair conformation of the pyranose ring. The presence of adenin-9-yl moiety in position 4 is evidenced by a downfield shift of the H-4 proton signal (1·45 ppm) and an upfield shift of the C-4 signal as compared with that of 1,6-anhydro-β-D-glucopyranose[15,19].

In the spectrum of compound *VII*, obtained by reduction of the epoxide *II*, the value of $J(6, 6')$ ($-7{\cdot}5$ Hz) proves preservation of the 1,6-anhydro ring. Upfield shifts of the H-2 and H-2′ protons (δ 2·27 and 1·78; $J(2, 2') = -15{\cdot}3$ Hz) and of the C-2 carbon atom (δ 36·18) of the carbohydrate moiety confirm the presence of a CH_2 group in position 2. The coupling constants of the pyranose ring protons agree with those described[16] for 1,6-anhydro-2-deoxy-β-D-*arabino*-hexopyranose.

The signals due to two CH_2—O groups ($J_{gem} \sim -11$ Hz), together with the corresponding number of hydroxyl protons (5 and 4, respectively), prove the absence of the 1,6-anhydro ring in compounds *IX* and *XI*. The *gluco*-configuration of compound *IX* follows from its preparation; it is confirmed by the marked similarity of vicinal coupling constants with those described[20] for D-glucitol, indicating also a similar preferred conformation. Comparison of ^{13}C chemical shifts for compound *IX* shows again a marked upfield shift of the C-4 signal ($-14{\cdot}7$ ppm) and only small upfield shifts of other carbon signals ($\leqq 0{\cdot}8$ ppm).

The presence of a CH_2 group in position 2 in compound *XI* is proven by the upfield shifted H-2 and H-2′ signals (δ 1·27 and 0·99), and by the coupling constant $J(2, 2') = -13{\cdot}9$ Hz. The ^1H NMR spectrum of the remaining part of the molecule is very similar to that of the compound *IX*. The structure of compound *XI* is further supported by the ^1H and ^{13}C NMR spectra of its pentaacetate *XIII* and tetraacetate *XII* (with free adenine amino group).

All the compounds prepared in this study afforded satisfactory elemental analyses and ultraviolet absorption spectra characteristic of adenine derivatives. Their mass spectra exhibited characteristic molecular ions and expected fragments BH, BH_2 (ions m/z 135 and 136, typical for adenine derivatives).

EXPERIMENTAL

Unless stated otherwise, the solutions were evaporated at 40°C/2 kPa and the compounds were dried at 25 Pa over phosphorus pentoxide. The melting points were determined on a Boëtius block and are uncorrected. Optical rotations were measured on a Bendix–Ericsson ETL 143 A automatic polarimeter at 20°C. Thin-layer chromatography was carried out on Silufol UV 254 sheets in the systems: S1 chloroform–methanol (9 : 1), S2 chloroform–methanol (4 : 1), S3 chloroform–methanol (1 : 1), and S4 ethyl acetate–acetone–ethanol–water (15 : 3 : 4 : 3). Preparative column chromatography was performed on silica gel Silpearl UV 254 (Kavalier). The adenine-containing products were deionized on a column of Dowex 50X8 (H^+-form) and, after washing out the salts with water, eluted with 2·5% aqueous ammonia; detection at 254 nm with a Uvicord 4 701 A (LKB) instrument. The UV-absorbing eluate was collected. The UV absorption spectra were recorded in aqueous solutions at pH 2 on a Pye Unicam 8 800 spectrometer.

Proton and ^{13}C NMR spectra of compounds *I*, *II*, *IV*—*VII*, *IX*, *XI*—*XIII* (Tables I and II) were taken on a Varian XL-200 (200 MHz and 50·3 MHz, respectively) FT spectrometer in hexadeuterodimethyl sulfoxide at 30°C and are referenced to tetramethylsilane (for the ^{13}C NMR spectra $\delta(CD_3SOCD_3) = 39{\cdot}7$). The chemical shifts and proton coupling constants were obtained by first order analysis from the expanded spectra (2 Hz/cm), using resolution enhancement. When necessary, the interacting protons were determined by decoupling experiments; the hydroxyl protons were proven by D → H exchange with a few drops of CD_3COOD. The ^{13}C NMR spectra were measured with a broad-band proton decoupling. The carbon signals were classified according to the number of directly attached protons, using the APT pulse sequence[21]; this, together with their chemical shifts and comparison with the published data, enabled their assignment.

The mass spectra were obtained with a Varian MAT 311 instrument (ionizing electrons energy 70 eV, direct inlet at 150—230°C, ion source temperature 200°C), IR spectra were recorded on a PE 684 (Perkin–Elmer) spectrophotometer.

4-(Adenin-9-yl)-1,6:2,3-dianhydro-4-deoxy-β-D-mannopyranose (*II*)

A suspension of adenine (14·85 g, 0·11 mol) and sodium hydride (2·64 g, 0·11 mol) in dimethylformamide (400 ml) was stirred at 60°C for 1 h. Epoxide *I* (29·8 g, 0·10 mmol) was added and the mixture was stirred at 100°C for 13 h. The reaction was monitored by TLC in the system S2. The insoluble portion was filtered off and the filtrate taken down. The residue was codistilled with toluene (2 × 200 ml) and extracted with boiling chloroform (3 × 500 ml). The insoluble portion was then extracted with boiling methanol and the combined methanolic and chloroform extracts were stripped of the solvents in vacuo. The residue in a small amount of methanol was adsorbed on silica gel (50 ml) and chromatographed on a column of silica gel (400 ml). After elution of the starting epoxide *I* (4·45 g, 15%) with chloroform–methanol (19 : 1), the solvent ratio in the mobile phase was changed to 12 : 1. The product-containing fractions were combined, the solvents evaporated and the residue was crystallized from ethanol–ethyl acetate (3 : 1, 100 ml) with addition of light petroleum to turbidity (~20 ml). The separated product was collected, washed with light petroleum and dried at 25°C in vacuo; yield 10·05 g (38·5%) of *II*,

m.p. 253°C, $[\alpha]_D$ −38° (*c* 0·68, dimethyl sulfoxide). For $C_{11}H_{11}N_5O_3$ (261·2) calculated: 50·58% C, 4·24% H, 26·81% N; found: 50·44% C, 4·26% H, 26·52% N. IR spectrum ($CHCl_3$, cm^{-1}): 3 527, 3 413 ($\tilde{\nu}$(NH)), 3 016, 3 138 ($\tilde{\nu}$(C—H) of the adenine cycle), 1 630, 1 585. Mass spectrum: 261 [M^+], 232 (M − 29], 190 [M − 71], 135 [BH], 108. UV spectrum: λ_{max} 257 nm, ε_{max} 1 660.

2,4-Bis(adenin-9-yl)-1,6-anhydro-2,4-dideoxy-β-D-glucopyranose (*IV*)

The insoluble portion, separated from the reaction mixture in the preparation of compound *II* was filtered, washed with hot water and dissolved in hot 5% acetic acid (60 ml). The boiling solution was decolorized with charcoal (0·5 g), filtered, cooled and neutralized with 25% aqueous ammonia. After standing overnight in a refrigerator, the separated product *IV* was collected and washed with water; yield 3·35 g (8·5%), not melting up to 260°C. $[\alpha]_D$ −41° (*c* 1·75, dimethyl sulfoxide). For $C_{16}H_{16}N_{10}O_3$ (396·4) calculated: 48·48% C, 4·07% H, 35·34% N; found: 48·22% C, 4·04% H, 35·59% N. Mass spectrum: 262 (M − $C_5H_4N_5$], 260 [M − $C_5H_6N_5$], 174, 148, 136 [BH_2], 135 [BH]. UV spectrum: λ_{max} 258 nm, ε_{max} 31 800.

Reaction of Compound *I* with Two Equivalents of Adenine Sodium Salt

A suspension of adenine (2·7 g, 20 mmol) and sodium hydride (0·53 g, 22 mmol) in dimethylformamide (60 ml) was heated to 60°C. After 1 h, compound *I* (2·98 g, 10 mmol) was added and the reaction mixture was heated to 100°C for 15 h. The monitoring of the reaction by TLC in the system S1 proved the formation of compound *II* at the early stage of the reaction. The insoluble 2,4-bis(adenin-9-yl)-1,6-anhydro-2,4-dideoxy-β-D-glucopyranose (*IV*), which began to separate after 7 h, finally represented the only reaction product. The precipitate was collected, washed with dimethylformamide and ethanol, boiled with water and filtered. The product *IV* on the filter was washed with light petroleum and dried in vacuo at 20°C. Another portion of *IV* was obtained from aqueous mother liquor on standing in a refrigerator overnight. Yield 3 g of *IV* (76% based on *I*).

Reaction of Epoxide *II* with Adenine Sodium Salt

A suspension of adenine (176 mg, 1·3 mmol) and sodium hydride (31 mg, 1·3 mmol) in dimethylformamide (5 ml) was heated to 60°C for 1 h. Compound *II* (261 mg, 1 mmol) was added and the reaction mixture was heated to 100°C. The reaction was monitored by TLC in the system S1. After 3·5 h, compound *IV* was the sole reaction product. It was isolated as described in the preceding experiment; yield 162 mg (60·7%).

(4-Adenin-9-yl)-1,6-anhydro-4-deoxy-β-D-glucopyranose (*VI*)

A) Compound *II* (10·4 g, 40 mmol) was heated with 1M-NaOH (400 ml) to 100°C under argon. After 2 h, the hot reaction mixture was neutralized with Dowex 50X8 (H^+-form). The Dowex was filtered off, the filtrate taken down and the residue crystallized from water. On standing in a refrigerator, dihydrate of compound *VI* was obtained as tiny needles; yield 4·4 g (35%), m.p. 245—248°C, $[\alpha]_D$ −21° (*c* 0·58, 0·1M-HCl). For $C_{11}H_{13}N_5O_4.2\ H_2O$ (315·3) calculated: 41·86% C, 5·43% H, 22·19% N; found: 41·77% C, 5·20% H, 22·22% N. Mass spectrum: 279 [M^+], 234 [M − 45], 190, 136 [BH_2], 135 [BH]. UV spectrum: λ_{max} 258 nm, ε_{max} 16 400.

B) A mixture of compound *II* (0·5 g, 1·91 mmol) and 0·5M-H_2SO_4 (50 ml) was heated on a steam bath for 24 h. The reaction mixture was cooled to 20°C, neutralized with 25% aqueous

ammonia and set aside in a refrigerator for 24 h. The separated product *VI* was filtered and washed with ice-cold water, ethanol and ether. Yield 0·28 g (46·7%); further portion of the product was obtained after deionization of the mother liquor.

4-(Adenin-9-yl)-1,6-anhydro-2,4-dideoxy-β-D-*arabino*-hexopyranose (*VII*)

Compound *II* (7·5 g, 20·9 mmol) was refluxed with a suspension of lithium aluminium hydride (5·04 g, 66 mmol) in tetrahydrofuran (300 ml) for 3 h. The reaction mixture was cooled to room temperature and decomposed by addition of ethyl acetate (150 ml) and water (25 ml). After standing for 1 h, the supernatant was decanted and taken down. The sediment was freed of the remaining solvents in vacuo and mixed with water (300 ml). Dowex 50X8 (H^+-form) was gradually added until the solid completely dissolved. This mixture was applied onto a column of Dowex 50X8 (H^+-form, 300 ml). After washing with water to drop of conductivity of the eluate to the original value, the adenine derivatives were eluted with 2·5% aqueous ammonia. The fractions were taken down in vacuo and the residue was combined with the material obtained from the above supernatant. This mixture was dissolved in aqueous ethanol, decolorized with charcoal at the boil and taken down. The residue was chromatographed on a column of silica gel (350 ml) in chloroform–methanol (15 : 1) to give compound *VII* which was crystallized from aqueous ethanol; yield 1·73 g (22·8%), m.p. 240—242°C, $[\alpha]_D$ −15° (*c* 0·68, 0·1M-HCl). For $C_{11}H_{13}N_5O_3.1/2\,H_2O$ (272·3) calculated: 48·52% C, 5·18% H, 25·72% N; found: 48·51% C, 4·99% H, 26·07% N. IR spectrum (Nujol, cm^{-1}): 3 384, 3 328, 3 140, 2 948, 2 919, 2 852, 1 690, 1 611. Mass spectrum: 263 [M^+], 190, 174, 162, 148, 136 [BH_2], 135 [BH], 108. UV spectrum: λ_{max} 257 nm, ε_{max} 14 400.

4-(Adenin-9-yl)-4-deoxy-D-glucitol (*IX*)

Compound *VI* (350 mg, 1·25 mmol) was heated with 36% hydrochloric acid (4·5 ml, 52·2 mmol) to 60°C for 2 h under argon. The reaction mixture was neutralized with sodium hydrogen carbonate and diluted with water (10 ml). A solution of sodium borohydride (150 mg, 3·97 mmol) in water (3 ml) was slowly added dropwise under stirring. After 24 h, Dowex 50X8 (H^+-form) was added to acid reaction and the mixture was deionized on a column of Dowex 50 (100 ml) as described above. A solution of the deionized product *IX* was concentrated and chromatographed on a column of silica gel (15 g), first in chloroform–methanol (3 : 2) until the starting compound *VI* was eluted, and then in chloroform–methanol (1 : 1). The product fractions were combined and the solvents evaporated to afford 162 mg (43·1%) of amorphous hydroscopic compound *IX*, $[\alpha]_D$ +5° (*c* 0·61, 0·1M-HCl). IR spectrum (Nujol), cm^{-1}: 3 326, 3 110, 2 923, 1 648, 1 605, 1 461. Mass spectrum: 299 [M^+], 238, 178, 136 [BH_2], 135 [BH], 108. UV spectrum: λ_{max} 258 nm, ε_{max} 16 700.

Further Attempts to Cleave the 1,6-Anhydro Bond in *VI*

A) Hydrolysis with dilute sulfuric acid. Compound *VI* (10 mg) was heated with dilute sulfuric acid (1 ml, concentrations 0·05—0·5 mol l^{-1}) to 100°C under argon in sealed ampoules. After 24 h, all the ampoules contained only the starting compound *VI*. Prolonged heating with 3M-H_2SO_4 led to complete degradation of the compound.

B) Acetolysis. A mixture of compound *VI* (100 mg, 0·316 mmol), acetic anhydride (23 ml) and trifluoroacetic acid (1·7 ml) was allowed to stand at room temperature for 24 h. After evaporation, the reaction product was heated with 0·1M-CH_3ONa in methanol. According to TLC, the mixture contained solely the starting compound *VI*.

C) Cleavage with bromotrimethylsilane. A mixture of compound *VI* (50 mg), hexamethyldisilazane (3 ml) and a trace of ammonium sulfate was stirred at 130°C for 2 h. After removal of hexamethyldisilazane in vacuo, the reaction mixture was mixed with dichloromethane (2 ml), and bromotrimethylsilane (0·6 ml) was added. The reaction mixture was heated to 70°C for 2·5 h under reflux condenser. After evaporation in vacuo and hydrolysis, samples of the mixture contained only the starting compound.

D) Cleavage with hydrogen bromide. A mixture of compound *VI* (279 mg, 1 mmol), acetic anhydride (1·5 ml) and 30% hydrogen bromide in acetic acid (3 ml) was set aside in a sealed ampoule under argon for 24 h at room temperature. As shown by TLC in S2, the reaction mixture contained only the peracetate of the starting compound *VI*. Even after standing at room temperature for 15 days or heating to 70°C for 3 h, no further product was detected. The reaction mixture was taken down and the residue was deacetylated by heating with 0·1M-CH_3ONa in methanol. The starting compound *VI* was detected as the only UV-absorbing product.

4-(Adenin-9-yl)-2,4-dideoxy-D-*arabino*-hexitol (*XI*)

A solution of 2-deoxy derivative *VII* (1·64 g, 6·24 mmol) in 0·2M-HCl (164 ml, 32·86 mmol) was heated under argon to 60°C for 5 h and then set aside at room temperature for 24 h. The reaction was monitored by TLC in the system S4. After neutralization with sodium hydrogen carbonate, the reaction mixture was added dropwise to a stirred aqueous solution of sodium borohydride (4·93 g, 130 mmol) and allowed to stand at room temperature for 24 h. The solution was filtered, acidified with acetic acid and poured onto a column of Dowex 50X8 (H^+-form, 250 ml). After washing out the salts with water, the product was eluted with 2·5% aqueous ammonia. The UV-absorbing eluate was taken down in vacuo and the residue was chromatographed on silica gel (100 g) in the system S4. Yield 1·28 g (72·5%) of amorphous product *XI*, $[\alpha]_D$ +45° (*c* 0·38, H_2O). Mass spectrum: 283 [M^+], 238, 178, 136 [BH_2], 135 [BH], 108. UV spectrum: λ_{max} 257 nm, ε_{max} 13 400.

1,2,3,5,6-Tetra-O-acetyl-4-(adenin-9-yl)-2,4-dideoxy-D-*arabino*-hexitol (*XII*)

Acetic anhydride (1·02 g, 10 mmol) was added to a solution of 4-(adenin-9-yl)-2,4-dideoxy-D-*arabino*-hexitol (*XI*; 283 mg, 1 mmol) in pyridine (5 ml). After standing at room temperature for 24 h, methanol (4 ml) was added, the reaction mixture was taken down and the residue was dissolved in chloroform. The chloroform solution was washed with water, dried over sodium sulfate, the solvent evaporated and the product crystallized from chloroform–ethanol (2 : 1), yielding 223 mg (49·4%) of tetraacetate *XII*, m.p. 210–213°C. Chromatography of the mother liquors afforded 30 mg (6·1%) of 4-(adenin-9-yl)-2,4-dideoxy-D-*arabino*-hexitol pentaacetate (*XIII*). For $C_{19}H_{25}N_5O_8$ (451·4) calculated: 50·55% C, 5·88% H, 15·51% N; found: 50·57% C, 5·56% H, 15·20% N. Mass spectrum: 451 [M^+], 392, 178, 136 [BH_2], 95, 43.

REFERENCES

1. Holý A.: Chem. Listy *81*, 461 (1987).
2. DeClercq E., Descamps J., De Somer P., Holý A.: Science *200*, 563 (1978).
3. Holý A., Merta A., Veselý J., Votruba I.: Chem. Listy *79*, 623 (1985).
4. De Clercq E., Holý A.: J. Med. Chem. *28*, 282 (1985).
5. De Clercq E., Holý A., Votruba I.: Collect. Czech. Chem. Commun. *50*, 245 (1985).
6. Cantoni G. L., Chiang P. E., Guranowski A., Montgomery J. A.: Biochemistry *20*, 110 (1981).
7. Holý A.: Collect. Czech. Chem. Commun. *49*, 2148 (1984).

8. De Clercq E., Holý A., Votruba I.: Collect. Czech. Chem. Commun. *50*, 262 (1985).
9. Holý A.: Collect. Czech. Chem. Commun., in press.
10. Černý M., Gut T., Pacák J.: Collect. Czech. Chem. Commun. *26*, 2542 (1961).
11. Černý M., Staněk J. jr: Adv. Carbohydr. Chem. Biochem. *34*, 23 (1977).
12. Černý M., Černý I., Trnka T.: Carbohydr. Res. *67*, 33 (1978).
13. Luger P.: Unpublished results.
14. Seib P. A.: J. Chem. Soc. *14*, 2552 (1969).
15. Buděšínský M., Trnka T., Černý M.: Collect. Czech. Chem. Commun. *44*, 1949 (1979).
16. Buděšínský M., Černý M., Trnka T., Vašíčková S.: Collect. Czech. Chem. Commun. *44*, 1965 (1979).
17. Paulsen H., Koebernick H.: Chem. Ber. *109*, 104 (1976).
18. Trnka T., Černý M., Buděšínský M., Pacák J.: Collect. Czech. Chem. Commun. *40*, 3038 (1975).
19. Trnka T., Černý M., Shmyrina A. Ya., Shashkov A. S., Sviridov A. F., Chizhov O. S.: Carbohydr. Res. *76*, 39 (1979).
20. Hawkes G. R., Lewis D.: J. Chem. Soc., Perkin Trans. 2 *1984*, 2073.
21. Le Cocq C., Lallemand J.-Y.: J. Chem. Soc., Commun. *1981*, 150.

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