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ISOLATION AND IDENTIFICATION OF PRODUCTS IN THE REACTION OF 2-ACETAMIDO-3,4,6-TRI-*O*-ACETYL-1,5-ANHYDRO-2-DEOXY-D-*arabino*-HEX-1-ENITOL WITH THEOPHYLLINE*

NEVENKA PRAVDIĆ** AND BORIS DANILOV
Department of Organic Chemistry and Biochemistry, "Ruđjer Bošković" Institute, 41001 Zagreb, Croatia (Yugoslavia)

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ABSTRACT

Fusion of 2-acetamido-3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol with theophylline, in the presence of boron trifluoride etherate as the catalyst, caused condensation to occur. This reaction afforded a variety of products of nucleosidic character, which were successively separated by repeated chromatography on silica gel. The structures of the products were determined, on the basis of X-ray crystallographic analysis (for three compounds) and by means of n.m.r.-spectral data and mass spectrometry, as the following: 7-(2-acetamido-4,6-di-*O*-acetyl-2,3-dideoxy-β-D-*erythro*-hex-2-enopyranosyl)theophylline, the corresponding α- and β-D-*threo* derivatives, and 7-(2-acetamido-6-*O*-acetyl-2,3-dideoxy-α-D-*threo*-hex-2-enopyranosyl)theophylline and its β anomer.

In addition to these 2′,3′-unsaturated nucleosides having the base linked at C-1′, three products of a new type, having the base attached at C-4′, were also isolated: 7-(methyl 2-acetamido-6-*O*-acetyl-2,3,4-trideoxy-β-D-*erythro*-hex-2-enopyranosid-4-yl)theophylline, and the corresponding α-D-*threo* and α-D-*erythro* isomers.

The correlation of the data obtained by X-ray structure analysis and proton nuclear magnetic spectroscopy, together with their application for the determination of configuration and conformation of these compounds, are discussed. It appears that the ^{1}H-n.m.r. data alone do not suffice for unambiguous and correct structure determination for these classes of compounds.

INTRODUCTION

Interest in the synthesis of unsaturated nucleosides is connected with recognition of the structure of the nucleoside antibiotic[1,2] Blasticidin S. Owing to the biochemical significance of unsaturated nucleosides[3–5], numerous papers describing the direct

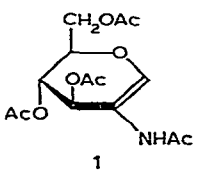
*Unsaturated Amino Sugars in the Preparation of a New Type of Nucleoside, Part I.
**Present address: Referral Centre of the University of Zagreb, P.O. Box 327, 41001 Zagreb, Yugoslavia.

utilization of unsaturated sugars in the synthesis of nucleosides have been published. The acid-catalyzed, fusion procedure is the method most frequently employed in the synthesis. It usually gives complex mixtures of reaction products[6–20], from which products of two general types have been isolated: (*i*) 2′,3′-unsaturated nucleosides with the base linked at C-1′, and (*ii*) 1′,2′-unsaturated isomers having the base attached at C-3′.

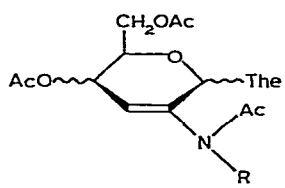
We have briefly reported[21] the first application of an unsaturated amino sugar [2-acetamido-3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol (**1**)] in the preparation of nucleosides. In the fusion reaction of **1** with theophylline as the base, a variety of products of nucleosidic character was formed. One of these products was separated, and, by X-ray crystallographic analysis, its structure was determined[21] to be one of a new type, *i.e.*, a 2′,3′-unsaturated nucleoside with the base linked at C-4′ of the carbohydrate moiety. We now report the isolation and identification of all of the products formed in the aforementioned reaction; in the following paper[22], evidence is presented bearing upon the mechanism of the reaction involved.

RESULTS

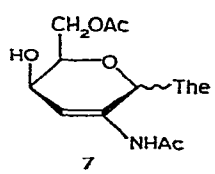
2-Acetamido-3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol (**1**), obtained by selective *N*-deacetylation[23] from the corresponding 2-(*N*-acetylacetamido) derivative[24,25], was used as the starting material. A mixture of **1** and theophylline was fused in the presence of a catalytic amount of boron trifluoride etherate for 30 min at 85–90°, to afford the crude product as a multicomponent mixture. It was successively separated by repeated chromatography on columns of silica gel; elution with various solvent-systems led to the isolation of products **2**, **3**, and **7**. Under these conditions, ~15% of the starting material **1** was recovered. When the fusion reaction



1



β-D-*erythro* 2 R = H 4 R = Ac
α-D-*threo* 3 R = H 5 R = Ac
β-D-*threo* 3 R = H 6 R = Ac
The = theophylline



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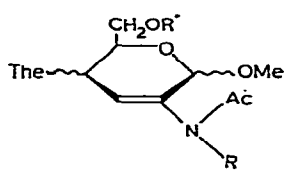

β-D-*erythro* 8 R = H, R′ = Ac 11 R = R′ = H 14 R = R′ = Ac
α-D-*threo* 9 R = H, R′ = Ac 12 R = R′ = H 15 R = R′ = Ac
α-D-*erythro* 10 R = H, R′ = Ac 13 R = R′ = H

TABLE I

N.M.R. PARAMETERS: CHEMICAL SHIFTS AND COUPLING CONSTANTS FOR COMPOUNDS 2–15; MEASUREMENTS MADE AT 60 MHz

Compound	*Chemical shifts (τ values)*										*Coupling constants (Hz)*		*Solvent*[a]
	NH[b]	*H-8*	*H-1'*	*H-3'*	*H-4'*	*H-5'*, H-*6'*,*6''*	*NCH*$_3$	*OCH*$_3$	*NAc and OAc*	*OH*[b]	$J_{3',4'}$	$J_{4',5'}$	
2	0.56 s	1.97 s	3.68 s	3.31 d	4.52 d	5.73 s	6.59, 6.80	—	7.96, 7.98, 8.01	—	6.0	0	A
3-α	1.78 s	2.20 s	3.47 s	3.09 d	4.10 dd	5.3–6.2 m	6.39, 6.59	—	7.80, 7.89, 7.97, 8.01	—	6.2		B
3-β	1.89 s	2.12 s	3.62 s	3.22 d							3.0		
4	—	2.00 s	3.55 s	3.68 d	4.24 d	5.4–5.8 m	6.41, 6.62	—	7.48[c] 7.79, 7.85	—	6.0		B
5	—	1.99 s	3.49 s	3.70 d	3.98 dd	5.3–6.2 m	6.38, 6.58	—	7.51[c] 7.82, 8.00	—	6.1	3.0	B
6	—	2.29 s	3.62 s	3.94 d	~4.2 m	5.7–6.0 m	6.42, 6.62	—	7.52[c] 7.83, 7.94	—	2.1		B
7-α	0.64 s	2.02 s	4.53 s	3.40 d	4.40 m	5.2–6.4 m	6.58, 6.78	—	8.02, 8.04, 8.07, 8.12	2.93 d	6.4		A
7-β	0.96 s	1.84 s	4.66 s	3.65 d						2.98 d	2.8		
8	0.78 s	2.04 s	4.93 s	3.48 d	4.48 d	5.76 s	6.60, 6.78	6.60	7.95, 8.00	—	6.0	0	A
9	3.03 s	2.35 s	4.93 s	3.31 d	4.24 dd	5.4–6.3 m	6.44, 6.64	6.53	7.92, 8.04	—	6.4	3.1	B
10	3.06 s	2.25 s	5.00 s	3.35 d	4.11 dd	5.6–6.0 m	6.42, 6.62	6.50	7.91, 7.98	—	2.5	9.0	B
11	0.80 s	2.04 s	5.00 s	3.52 d	4.58 dd	5.8–6.1 m	6.59, 6.79	6.59	8.03		5.8		A
12	0.68 s	2.10 s	4.93 s	3.48 d	4.47 dd	5.7–6.2 m	6.60, 6.78	6.60	8.03	5.39 t	6.5	4.0	A
	3.02 s	2.37 s	4.93 s	3.31 d	4.36 dd	5.6–6.3 m	6.44, 6.62	6.51	7.90		6.5	3.6	B
13	0.80 s	2.00 s	5.02 s	3.70 d	4.44 dd	5.6–6.2 m	6.58, 6.78	6.58	8.05	5.35 t	2.1	9.8	A
	2.79 s	2.20 s	5.00 s	3.38 d	4.17 dd	6.1–6.3 m	6.43, 6.60	6.52	7.91		2.1	9.5	B
14	—	1.98 s	4.96 s	3.87 d	4.26 dd	5.3–5.9 m	6.43, 6.63	6.53	7.54[c] 7.81	—	6.0	~1	B
15	—	1.98 s	4.92 s	3.88 d	4.10 dd	5.3–6.2 m	6.42, 6.62	6.53	7.60[c] 8.01	—	6.0	3.8	B

[a]Solvent: A, dimethyl sulfoxide-d_6; B, chloroform-*d*. [b]Signal removed by D_2O exchange. [c]Six-proton signal corresponding to two NAc groups.

was prolonged to a period of 2 h, similar processing of the crude nucleosidic mixture resulted in the separation of several products, of two different classes: in addition to compounds **2**, **3**, and **7**, already mentioned, nucleosides of another type (compounds **8–10**) were isolated.

Nucleoside **2** was assigned the structure 7-(2-acetamido-4,6-di-*O*-acetyl-2,3-dideoxy-β-D-*erythro*-hex-2-enopyranosyl)theophylline. The n.m.r. spectrum (see Table I) clearly showed the presence of three acetyl groups, indicating the loss of one acetoxyl group from the sugar moiety; the doublet at τ 3.31, a sharp singlet at τ 3.68, and a doublet at 4.52, were assigned to H-3′, H-1′, and H-4′, respectively. The facts that the signal of H-4′ is a doublet (with the same coupling constant as that for H-3′) and that there is a sharp, three-proton singlet (H-5′ and 2 H-6′) at τ 5.73 indicate that the $J_{4',5'}$ value is practically zero. This is in agreement with the observation[26,27] that the $J_{4,5}$ value for galactosides is usually abnormally small, and might suggest the D-*threo* configuration for the product **2**. Nevertheless, the D-*erythro* configuration in an alternative conformation seems to be a more plausible structure. The β-D-anomeric configuration of **2** was assigned on the basis of the results of this investigation, as well as on the basis of the pronounced tendency of β-aldohex-2,3-enoses[28–31] to adopt a conformation that would place the C-1 substituent in *quasi*-axial orientation.

The second product **3**, was found to be an isomer of **2**. Its n.m.r. spectrum revealed two sets of resonances for the NH, the anomeric, and the vinyl protons, and for H-8 of the base. These data suggested that **3** is a mixture of two components; the ratio of the components differed from batch to batch, but one of them always preponderated. The mixture proved to be almost inseparable by standard, chromatographic methods (column and preparative, thin-layer chromatography). However, when **3** was subjected to reaction with isopropenyl acetate, the two *N*-acetyl-acetamido derivatives formed were successfully separated, and their structures were determined (as described later herein). This permitted tentative assignment of the two components in **3** as 7-(2-acetamido-4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*threo*-hex-2-enopyranosyl)theophylline (**3**-α) and its β anomer (**3**-β).

The product eluted last from the column of silica gel was designated as **7**. From its n.m.r. spectrum, it is evident that **7** is a mixture, and that each component has one free hydroxyl group; as the hydroxyl hydrogen atoms appear as doublets, their location should be at C-4′. That the nucleoside **3** is a precursor in the formation of **7** was proved by the deliberate transformation of **3** into **7** through the action of silica gel. Acetylation of **7** afforded the di-*O*-acetyl derivative **3**, thus confirming its structure. This allowed assignment to the two components of **7** the structure 7-(2-acetamido-6-*O*-acetyl-2,3-dideoxy-D-*threo*-hex-2-enopyranosyl)theophylline (**7**-α and **7**-β).

The products **8–10**, which were encountered only in the crude product-mixtures obtained from the fusion for longer reaction-times, showed in their n.m.r. spectra (see Table I) the presence of an unexpected, three-proton singlet in the region of τ 6.50–6.60. The separation of these three products, which were found to be isomeric, is a tedious process. However, once obtained in pure form, compounds **8** and **9**

were prepared as crystals of good quality for crystallographic analysis. On the basis of the X-ray crystal structure determination[32], compound **8** was designated as 7-(methyl 2-acetamido-6-*O*-acetyl-2,3,4-trideoxy-β-D-*erythro*-hex-2-enopyranosid-4-yl)-theophylline, whereas the major product in this reaction, compound **9** was found[21,33] to be 7-(methyl 2-acetamido-6-*O*-acetyl-2,3,4-trideoxy-α-D-*threo*-hex-2-enopyranosid-4-yl)theophylline. The third isomer of this series was identified as 7-(methyl 2-acetamido-6-*O*-acetyl-2,3,4-trideoxy-α-D-*erythro*-hex-2-enopyranosid-4-yl)theophylline (**10**).

Deacetylation of compounds **8**–**10** with sodium methoxide readily removed the *O*-acetyl group, to give crystalline products **11**–**13**, respectively. As the separation of the α-D-*erythro* compound **10** from the fusion-product mixture afforded pure **10** in only very low yield (~2%), the mixture containing **9** and **10** was deacetylated. The resulting compounds, **12** and **13**, were readily separable, and pure **13** was then used for the preparation of **10**. The X-ray crystal structure determined[34] for **13** established its molecular structure as 7-(methyl 2-acetamido-2,3,4-trideoxy-α-D-*erythro*-hex-2-enopyranosid-4-yl)theophylline. The n.m.r. spectra of compounds **11**–**13**, recorded for solutions in dimethyl sulfoxide, showed the hydroxyl hydrogen atoms as triplets, supporting the C-6′ position of the acetoxyl groups in compounds **8**–**10**.

In the course of this work, the *N*-acetylacetamido derivatives (**4**–**6**, **14**, and **15**) were synthesized through the action of isopropenyl acetate on compounds **2**, **3**, **8**, and **9**, respectively. Two products that were formed in the reaction from **3**, were, on the basis of their n.m.r. spectra, identified as the α-D-*threo* (**5**) derivative and the β-D-*threo* (**6**).

DISCUSSION

Nucleosides containing a 2-acetamido group at the vinyl position of a carbohydrate moiety, obtained as the products in the condensation of the unsaturated amino sugar **1** with theophylline, belong to two structurally different groups. First, compounds **2**, **3**-α, and **3**-β are of the type (2′,3′-unsaturated nucleosides having the base linked at C-1′) already encountered in the reactions using simple, unsaturated sugars in the direct synthesis of nucleosides[6–19]. They could also be correlated with the 2-acetamido-2,3-unsaturated glycosides that are produced on treatment of appropriately substituted, unsaturated amino sugars with various nucleophiles[27,35]. In addition to compounds **2** and **3**, which may, for short, be named 7-C-1′-theophylline derivatives, fusion for 30 min also afforded products **7**-α and **7**-β. These compounds are of essentially the same type, being generated from **3**-α and **3**-β, respectively, by the loss of one acetyl group, at O-4′. Our understanding of this partial *O*-deacetylation is as yet highly deficient: it might occur in the course of the fusion or during the chromatography on silica gel. Nevertheless, it indicates a certain lability of the C-4′ linkage.

Second, compounds **8**, **9**, and **10** have an unusual structure; they are members of a completely new type of nucleoside, with 2′,3′-unsaturation, the base linked at

C-4′, and a methoxyl group glycosylically bonded. As **8–10** were isolated only from the condensations that were conducted for 2 h at the fusion temperature, it is suggested that these compounds are secondary products, and that the methoxyl group originates from methanol used in the processing, because, when ethanol was used instead of methanol, products **8–10** were not isolated. However, the formation of an ethyl glycoside corresponding in its structure to the isomeric **8–10** was observed. Although this compound was not isolated in pure form, its presence in samples of the nucleosidic mixture was demonstrated by means of mass spectrometry. Evidence bearing upon the formation of nucleosides having the base attached at C-4′ (7-C-4′-theophylline derivatives), and on the mechanism of the reaction involved, is presented in the accompanying paper[22].

The basis for structure determination of all of the compounds described herein are the results of X-ray structure analyses[32–34] obtained for compounds **8**, **9**, and **13**. The correlation of the data from this method and those from the proton-n.m.r. spectra (see Table I) will be presented first.

The α-D-*threo* derivative **9** in crystalline form was found[33] to exist in the $^{o}H_5$ conformation, the consequence of which is a *quasi*-equatorial orientation for the allylic H-4′ atom, and an axial orientation for H-5′. This is in full accord with the n.m.r. data: the magnitude of $J_{3',4'}$ (6.4 Hz) corresponds to vinyl-allylic proton coupling with the allylic proton in *quasi*-equatorial orientation[15,36], whereas the value (3.1 Hz) found for $J_{4',5'}$ fits the *quasi*-equatorial/axial relationship well.

Compound **13**, *i.e.*, the *O*-deacetylated derivative of isomer **10**, was established[34] as being of the α-D-*erythro* configuration, adopting (in crystalline form) the same ($^{o}H_5$) conformation. The n.m.r. data from the spectra of compounds **13** and **10** offer full support for such a structure: the large value of $J_{4',5'}$ (9.5 Hz in the spectrum of **13**, and 9.0 Hz for **10**) is indicative of *quasi*-axial and axial orientation, respectively. The magnitude of $J_{3',4'}$ (2.1 and 2.5 Hz for compounds **13** and **10**, respectively), which is considered[36] to be characteristic of vinyl-allylic proton-coupling in the *erythro* series, reveals the *quasi*-axial orientation of the allylic proton.

The third compound examined on stereochemical grounds by using X-ray structure analysis, namely, isomer **8**, was found[32] to belong to the *erythro* series and to be of the β-D configuration. The conformation is $^{5}H_{o}$, a rather distorted half-chair, as the displacement of C-5 above the plane (defined with four contiguous atoms) is larger than that of the ring-oxygen atom below the plane. This alternative half-chair conformation places the C-1′ substituent in *quasi*-axial orientation. That the anomeric pair, **8** and **10**, exist in different half-chair conformations most probably arises from the anomeric effect[37], and it has already been established in the 2,3-unsaturated aldopyranose series[28–31]. The orientation of the bulky C-4′ substituent is *quasi*-axial, and that might[38] have considerable influence on the deformation of the conformation. The n.m.r. parameters are in accordance with the $^{5}H_{o}$ conformation found: the *quasi*-equatorial/equatorial relationship of H-4′ and H-5′, with ϕH-4′–H-5′ = 87° is adequately represented by $J_{4',5'}$ 0. The value of $J_{3',4'}$ (6.0 Hz) is in agreement with the *quasi*-equatorial orientation of the allylic proton. However, the n.m.r. parameters,

taken alone, could just as well clearly suggest the D-*threo* configuration, but in the stable, $^{o}H_5$ conformation. This should be taken as a warning that the n.m.r. data alone are insufficient for unambiguous, and correct, structure determination.

The most significant feature of the n.m.r. spectra of all of the 7-C-4′-theophylline derivatives **8–15** is a considerable, upfield shift of the anomeric protons. The singlets of H-1′ are found in the region τ 4.9–5.0. It seems plausible to assume that this unusual effect might be connected with the existence of the C–N linkage at C-4′. In a study published recently[39], the n.m.r. spectra of seven compounds of the *threo* and *erythro* series having a phthalimido group attached at C-4 (*e.g.*, methyl 2,3,4,6-tetradeoxy-4-phthalimido-α-DL-*threo*-hexopyranoside) were reported. The anomeric protons in the spectra of these compounds were also observed as high-field signals, in the region τ 4.7–5.1.

The n.m.r. parameters of the 7-C-1′-theophylline derivatives (see Table I) will now be briefly discussed. The anomeric protons in the spectra of compounds **2–6** appear as singlets in the region τ 3.5–3.7. In the spectra of various 2′,3′-unsaturated nucleosides having the base linked at C-1′, described in papers by Garcia-Munoz *et al.*[11,16] and others[10,15,18], the doublets of H-1′ occur at τ 3.1–3.5. The presence of an acetamido group at C-2 in our compounds might give rise to the upfield shift observed. The doublets of H-3′ and H-4′ in the spectra of the products of this group are revealed in the same region as the signals of these protons observed in the spectra of several 2-acetamido-2,3-dideoxyglycosyl derivatives[27,35], as well as in the spectra of numerous 2′,3′-unsaturated nucleosides[7,10,11,16,18,40].

Because a crystal-structure determination was not performed for either of the 7-C-1′-theophylline derivatives, their proton n.m.r. data will be used in the structure assignment, together with some observations gained through correlation of n.m.r. and X-ray structure-analysis data described earlier herein for the 7-C-4′-theophylline series.

The n.m.r. spectra of the three isomeric compounds **2**, **3-α**, and **3-β** did not reveal a large $J_{4',5'}$ value. This fact is very important and informative; due to it, the existence of an α-D-*erythro* derivative, which should normally favor the $^{o}H_5$ conformation, is excluded. Therefore, the compounds in our hands should be of the β-D-*erythro*, α-D-*threo*, and β-D-*threo* configurations. Starting with the assumption that the β anomers would adopt the less stable, $^{5}H_{o}$ conformation, as is the case with the β-D-*erythro* derivative **8**, the following structures could be ascribed for the compounds: **2** and **4**, β-D-*erythro*-$^{5}H_{o}$; **3-α**, **5**, and **7-α**, α-D-*threo*-$^{o}H_5$; and **3-β**, **6**, and **7-β**, β-D-*threo*-$^{5}H_{o}$. Thus, *quasi*-equatorially oriented, allylic protons in compounds **2**, **3-α**, **4**, **5**, and **7-α** give rise to $J_{3',4'}$ values of 6.0–6.4 Hz, whereas compounds **3-β**, **6**, and **7-β** having *quasi*-axially oriented, allylic protons reveal, in their n.m.r. spectra, $J_{3',4'}$ values of 2.1–3.0 Hz, and this is in agreement with the theoretical prediction[36].

It is well known that some 2,3-unsaturated aldenoses[28,29] and some unsaturated nucleosides[16] do not conform to Hudson's isorotation rule; this has also been found valid in the amino-nucleoside series. Two α anomers, such as **10** and **13**, show a

negative, optical rotation. However, in both cases, the rotations of these α anomers are less negative than those of their β anomers, **8** and **11**.

The u.v. spectra of each of the nucleosides **2**, **4–6**, and **8–15** show λ_{max} near 274 nm, demonstrating that they are all 7-substituted[41] theophyllines.

In conclusion, it should be noted that the fusion procedure, although extremely complex, seems to be the only method applicable for the preparation of nucleosides containing unsaturated amino sugars: attempts to reach this class of compounds by an indirect route failed[42].

EXPERIMENTAL

General methods. — Specific rotations were measured at 20–24°. T.l.c. was conducted on plates (5 × 10 cm) of silica gel F 254 (E. Merck) in the following solvent-systems: *A*, 9:1 ether–methanol; *B*, 5:5:2 chloroform–acetone–methanol; *C*, 12:1 chloroform–methanol; *D*, 1:1 ethyl acetate–acetone; *E*, 5:1 ether–methanol; *F*, 12:1:2 chloroform–methanol–ether; and *G*, 19:1 ether–methanol, all ratios being v/v. The components were detected under u.v. light, or by spraying with 10% sulfuric acid and heating. Column chromatography was performed on silica gel (E. Merck; 0.05–0.20-mm particle size) with the solvent system specified. U.v.-spectral data were obtained with a Perkin–Elmer double-beam spectrophotometer (Model 124) for solutions in methanol. I.r. spectra were recorded with a Perkin–Elmer 137 Infracord instrument. The n.m.r. spectra were recorded at 60 MHz with Varian A-60*A* and EM-360 spectrometers, for solutions in the solvents specified, with tetramethylsilane as the internal standard. Theophylline (E. Merck, wasserfrei) was dried overnight at 110° before use.

Reaction of **1** *with theophylline.* — (*A*) *Fusion for 30 min.* A mixture of **1** (2.7 g, 8.2 mmol) and theophylline (740 mg, 4.1 mmol) was heated in an oil bath preheated at 85°. When the components were fused, boron trifluoride etherate (5 drops) was added, the mixture was stirred, and heating was continued for 30 min at 85–90°, the progress of the reaction being monitored by t.l.c. with solvent *A*. The dark melt was dissolved in chloroform (10 mL), the solution mixed with silica gel (4 g), the solvent allowed to evaporate overnight at room temperature, and the residue then applied to a column of silica gel (15 g) prepacked in solvent *B*. Irrigation of the column with the same solvent (~20 mL) eluted first a mixture of a few degradation products and some unreacted **1**, from which was recovered **1** (15%). Further elution with the same solvent (~60 mL) afforded crude product (2.3 g) in the form of a brown foam, whereas the theophylline was mostly retained on the column.

The crude product was dissolved in methanol (10 mL), and the solution was kept overnight at room temperature. The crystalline material deposited was removed by filtration, and washed with methanol, to give 218 mg (12%) of *7-(2-acetamido-4,6-di-*O*-acetyl-2,3-dideoxy-β-*D-erythro-*hex-2-enopyranosyl)theophylline* (**2**) in the form of fine needles; m.p. 231–232°, $[\alpha]_D$ −56.5° (*c* 0.75, pyridine); λ_{max} 210, 232, and 274 nm (ε_{mM} 19.50, 19.50, and 7.95).

Anal. Calc. for $C_{19}H_{23}N_5O_8$: C, 50.78; H, 5.16; N, 15.58. Found: C, 50.64; H, 5.30; N, 15.67.

The filtrate was evaporated to dryness *in vacuo*, the residue dissolved in chloroform (2 mL), and the solution mixed with silica gel (2 g). The dried material was applied to a column of silica gel (15 g) prepacked with solvent *A*. The column was eluted with solvent *A*, 25-mL fractions being collected. Fractions 5–10 were pooled and evaporated: 1.01 g of a two-component mixture which was further separated on a column of silica gel (90 g) with solvent *C*, 10-mL fractions being collected.

Fractions 23–47 contained chromatographically homogeneous material, and evaporation gave product **3** in the form of a stable, colorless powder (840 mg, 46%). From fractions 73–100, after trituration of the residue with methanol, crystalline material (109 mg, 7%) was obtained. The n.m.r. spectrum and chromatographic behavior in several solvent-systems identified this product as **7**, more fully described later on.

Prior to analysis, a sample of **3** was rechromatographed with solvent *D*, giving a friable glass that failed to crystallize, $[\alpha]_D$ −48.2° (*c* 0.36, chloroform).

Anal. Calc. for $C_{19}H_{23}N_5O_8$: C, 50.78; H, 5.16; N, 15.58. Found: C, 50.46; H, 5.45; N, 15.52.

(*B*) *Fusion for 2 h.* A mixture of **1** (4.6 g, 14 mmol) and finely powdered, dried theophylline (1.26 g, 7 mmol) was heated at 85° (oil bath temperature). Boron trifluoride etherate (7 drops) was added, and the mixture was then stirred and heated for 2 h at 85–95°. At the end of the reaction, several u.v.-absorbing spots were detectable, whereas the spot for the starting sugar **1** was absent. The melt was cooled to room temperature, dissolved in chloroform (180 mL), and the solution successively washed with saturated aqueous sodium hydrogencarbonate solution, and water, dried (sodium sulfate), and concentrated *in vacuo* to ~5 mL; this was mixed with silica gel (5 g), and dried. The crude mixture of products, adsorbed on the silica gel, was applied to a column of silica gel (25 g) prepacked in solvent *A*. The column was first eluted with the same solvent-system (400 mL), to remove the nucleosidic material. The first fractions (~100 mL), which contained degradation by-products of carbohydrate character, were discarded. Further elution of the column with solvent *E* (500 mL) eluted material of low solubility (440 mg), which was not identified.

In the fractions eluted with solvent *A*, crystalline material deposited. It was filtered off: 51 mg, 1.6%; its i.r. spectrum, m.p., and mixed m.p. identified it as **2**.

The filtrate was evaporated *in vacuo*, to give a foamy, yellow residue (1.53 g, 52%). It was treated with methanol (3 mL) without heating, and after being kept overnight at room temperature, it crystallized. The crystals (853 mg), which were removed, and washed with methanol*, appeared to be homogeneous by t.l.c. in several solvent-systems. However, it was actually a two-component mixture, which was successfully separated by treating it with chloroform; to 853 mg was added chloroform (4 mL), and a part that did not dissolve without heating was filtered off:

*Separation of the methanolic mother-liquor is described later in this section.

88 mg (3%), m.p. 247–249°, $[\alpha]_D$ −71.6° (*c* 0.76, pyridine); λ_{max} 210, 232, and 274 nm (ε_{mM} 19.50, 19.90, and 7.95). By X-ray crystallographic analysis, the structure of this compound was determined[32] to be *7-(methyl 2-acetamido-6-O-acetyl-2,3,4-trideoxy-β-*D-erythro-*hex-2-enopyranosid-4-yl)theophylline* (**8**).

Anal. Calc. for $C_{18}H_{23}N_5O_7$: C, 51.30; H, 5.50; N, 16.62. Found: C, 51.55; H, 5.51; N, 16.41.

After evaporation, the chloroform filtrate afforded crystals that were recrystallized from methanol, to give *7-(methyl 2-acetamido-6-O-acetyl-2,3,4-trideoxy-α-*D-threo-*hex-2-enopyranosid-4-yl)theophylline*[21,33] (**9**) in the form of large prisms: 585 mg (20%), m.p. 222–224°, $[\alpha]_D$ +52.2° (*c* 1.1, chloroform); λ_{max} 230 and 274 nm (ε_{mM} 23.70 and 9.10).

Anal. Calc. for $C_{18}H_{23}N_5O_7$: C, 51.30; H, 5.50; N, 16.62. Found: C, 51.04; H, 5.31; N, 16.52.

The methanolic mother-liquor was evaporated *in vacuo*, and the residue (650 mg) was chromatographed on a column of silica gel (65 g) with solvent *C*, 8-mL fractions being collected. Fractions 20–27 yielded semicrystalline material (157 mg); its n.m.r. spectrum showed it to be a mixture of compounds **9** and **3**.

Fractions 28–34 afforded another mixture (342 mg) which was rechromatographed on silica gel with the same solvent-system. Fractions containing homogeneous material, with mobility identical to that of compound **9**, were pooled, and evaporated, and to the residue were added a few drops of methanol: crystals, 57 mg (2%), m.p. 190–192°, mixed m.p. with a sample of **9** gave depression. Recrystallization from methanol gave pure *7-(methyl 2-acetamido-6-O-acetyl-2,3,4-trideoxy-α-*D-erythro-*hex-2-enopyranosid-4-yl)theophylline* (**10**). The i.r. and n.m.r. spectra were superposable on those of an analytical sample described later in this paper; the mixed m.p. was undepressed.

Transformation of **3** *into* **7**. — To a solution of **3** (120 mg) in methanol (5 mL) was added dry silica gel (0.5 g), and the mixture was kept for 10 days at room temperature with occasional stirring, and examination by t.l.c. in solvent *C*. Chloroform (15 mL) was then added, the silica gel was filtered off, and the filtrate evaporated to dryness. Trituration with methanol afforded crystalline **7** (54 mg, 5%), which was recrystallized from 2-methoxyethanol: m.p. 213–215°, $[\alpha]_D$ +3.2° (*c* 0.82, pyridine); ν_{max}^{KBr} 3400 (OH), 3300 (NH), 1760 (OAc), 1720 (C=O), 1620 (C=C), 1660 and 1550 (Amide I and II), and 1230 cm^{-1} (OAc).

Anal. Calc. for $C_{17}H_{21}N_5O_7$: C, 50.12; H, 5.19; N, 17.19. Found: C, 50.23; H, 4.93; N, 16.98.

A sample of **7** (95 mg) was acetylated with acetic anhydride in pyridine to give, after the standard processing, an oily product (119 mg). It was chromatographed on a column of silica gel (12 g) with solvent *C*. From fractions containing homogeneous material, the anomeric mixture **3** was obtained in the form of a colorless powder: quantitative yield, $[\alpha]_D$ −59.4° (*c* 1.0, chloroform). The n.m.r. spectrum was superposable on that of an authentic sample.

*7-(Methyl 2-acetamido-2,3,4-trideoxy-β-*D-erythro-*hex-2-enopyranosid-4-yl)-*

theophylline (**11**). — To a solution of **8** (120 mg) in hot methanol (50 mL) was added 0.1M sodium methoxide (10 mL), and the mixture was kept for 2 h at room temperature, the progress of the reaction being monitored by t.l.c. in solvent *C*. Dry Dowex-50 X-8 (H^+) ion-exchange resin was then added, and the suspension was stirred for 5 min, and filtered. The filtrate was evaporated, to afford a crystalline residue, which was recrystallized from methanol: m.p. 220–222°, $[\alpha]_D$ −201.1° (*c* 0.5, $CHCl_3$); ν_{max}^{KBr} 3400 (OH and NH), 1720 (C=O), 1620 (C=C), and 1660 and 1540 cm^{-1} (Amide I and II); λ_{max} 212, 232, and 274 nm (ε_{mM} 18.50, 17.75, and 7.95).

Anal. Calc. for $C_{16}H_{21}N_5O_6$: C, 50.65; H, 5.58; N, 18.46. Found: C, 50.43; H, 5.63; N, 18.17.

*7-(Methyl 2-acetamido-2,3,4-trideoxy-α-*D-threo-*hex-2-enopyranosid-4-yl)theophylline* (**12**). — (*A*) *From* **9**. Compound **12** was prepared as described for the preparation of **11**. The crystalline product (80%) was recrystallized from methanol: m.p. 229–231° (dec.), $[\alpha]_D$ +78.6° (*c* 0.57, $CHCl_3$); ν_{max}^{KBr} 3560 (OH), 3380 (NH), 1705 (C=O), 1610 (C=C), and 1660 and 1560 cm^{-1} (Amide I and II); λ_{max} 210, 232, and 275 nm (ε_{mM} 11.50, 10.50, and 4.00).

Anal. Calc. for $C_{16}H_{21}N_5O_6$: C, 50.65; H, 5.58; N, 18.46. Found: C, 50.88; H, 5.82; N, 18.34.

(*B*) *From samples of crude* **9** *containing isomeric* **10**. Heterogeneous material (310 mg), obtained from mother liquors after isolation of compound **9**, was treated with sodium methoxide as already described. After neutralization of the base with an ion-exchange resin, the filtrate was evaporated to dryness, and the crude residue was chromatographed on a column of silica gel (25 g) with solvent *C*, 4-mL fractions being collected.

Fractions 34–46 were pooled, and evaporated, to yield crystalline product (132 mg, 47%) which, after recrystallization from methanol (+ ether) afforded pure *7-(methyl 2-acetamido-2,3,4-trideoxy-α-*D-erythro-*hex-2-enopyranosid-4-yl)theophylline* (**13**): m.p. 180–182°, $[\alpha]_D$ −104.6° (*c* 0.6, $CHCl_3$); ν_{max}^{KBr} 3480 (NH), 3400 (OH), 1680 (C=O), 1600 (C=C), and 1640 and 1550 cm^{-1} (Amide I and II); λ_{max} 209, 234, and 275 nm (ε_{mM} 10.50, 8.80, and 3.80).

Anal. Calc. for $C_{16}H_{21}N_5O_6$: C, 50.65; H, 5.58; N, 18.46. Found: C, 50.32; H, 5.43; N, 18.36.

From fractions 48–57 was obtained crystalline product (56 mg, 20%); its n.m.r. spectrum and chromatographic behavior identified it as the isomer **12**.

Acetylation

A sample of **12** (100 mg) was acetylated with acetic anhydride and pyridine in the usual way, to give, after standard processing, crystalline product: 110 mg, quantitative yield. After recrystallization from methanol, pure **9** was obtained: the i.r. and n.m.r. spectra were indistinguishable from those of an authentic sample, and the mixed m.p. was undepressed.

Similarly, a sample of **13** (155 mg) was acetylated to give, after recrystallization from methanol, *7-(methyl 2-acetamido-6-*O*-acetyl-2,3,4-trideoxy-α-*D-erythro-*hex-2-*

enopyranosid-4-yl)theophylline (**10**): 113 mg (65%); m.p. 193–195°, $[\alpha]_D$ −45.7° (*c* 0.63, $CHCl_3$); ν_{max}^{KBr} 3400 (NH), 1720 (OAc), 1700 (C=O), 1610 (C=C), 1660 and 1550 (Amide I and II), and 1240 cm^{-1} (OAc); λ_{max} 210, 234, and 275 nm (ε_{mM} 10.60, 9.10, and 3.95). The mobility of a sample of **10** in t.l.c. in several solvent-systems could not be distinguished from that of a sample of isomeric **9**.

Anal. Calc. for $C_{18}H_{23}N_5O_7$: C, 51.30; H, 5.50; N, 16.62. Found: C, 51.31; H, 5.79; N, 16.46.

*7-[4,6-Di-*O*-acetyl-2-*(N*-acetylacetamido)-2,3-dideoxy-β-*D*-*erythro*-hex-2-enopyranosyl]theophylline* (**4**). — A solution of **2** (200 mg) in isopropenyl acetate (25 mL) containing *p*-toluenesulfonic acid (20 mg) was boiled under reflux for 2 h. The solvent was evaporated *in vacuo*, and the crude product was chromatographed on a column of silica gel (20 g) with solvent *A*. Fractions containing homogeneous material were pooled: 91 mg, 42%; crystallized from methanol, m.p. 148–150°, $[\alpha]_D$ −86.2° (*c* 0.69, $CHCl_3$); ν_{max}^{KBr} 1740 (OAc), 1700 (C=O), 1600 (C=C), and 1220 cm^{-1} (OAc); λ_{max} 210 and 274 nm (ε_{mM} 14.30 and 3.80).

Anal. Calc. for $C_{21}H_{25}N_5O_9$: C, 51.32; H, 5.13; N, 14,25. Found: C, 51.31; H, 5.41; N, 14.11.

Treatment of **3** *with isopropenyl acetate.* — A solution of **3** (300 mg) in isopropenyl acetate (15 mL) containing *p*-toluenesulfonic acid (20 mg) was boiled under reflux for 1 h; t.l.c. in solvent *C* revealed the presence of two new products. After evaporation of the solvent, the crude product was chromatographed on a column of silica gel (30 g) in solvent *F*, 4-mL fractions being collected.

Fractions 11–15 afforded oily material (204 mg), which, after trituration with cold methanol, gave crystalline *7-(4,6-di-*O*-acetyl-2-*(N*-acetylacetamido)-2,3-dideoxy-α-*D*-*threo*-hex-2-enopyranosyl)theophylline* (**5**): 17 mg (5%), m.p. 184–185°, $[\alpha]_D$ +10.9° (*c* 0.67, $CHCl_3$); ν_{max}^{KBr} 1740 (OAc), 1700 (C=O), 1605 (C=C), and 1220 cm^{-1} (OAc); λ_{max} 210 and 174 nm (ε_{mM} 14.70 and 3.80).

Anal. Calc. for $C_{21}H_{25}N_5O_9$: C, 51.32; H, 5.13; N, 14.25. Found: C, 51.61; H, 5.26; N, 14.02.

Fractions 16–21 contained a mixture of two products (114 mg). It was rechromatographed on silica gel with the same solvent system. Homogeneous fractions afforded the second product: *7-[4,6-di-*O*-acetyl-2-*(N*-acetylacetamido)-2,3-dideoxy-β-*D*-threo-hex-2-enopyranosyl]theophylline* (**6**) in the form of a stable foam: $[\alpha]_D$ −33.0° (*c* 0.63, $CHCl_3$); ν_{max}^{KBr} 1740 (OAc), 1700 (C=O), 1600 (C=C), and 1230 cm^{-1} (OAc); λ_{max} 212 and 274 nm (ε_{mM} 20.33 and 7.25).

Anal. Calc. for $C_{21}H_{25}N_5O_9$: C, 51.32; H, 5.13; N, 14.25. Found: C, 51.31; H, 5.41; N, 14.18.

*7-[Methyl 6-*O*-acetyl-2-*(N*-acetylacetamido)-2,3,4-trideoxy-β-*D*-erythro-hex-2-enopyranosid-4-yl]theophylline* (**14**) was obtained on treatment of compound **8** with isopropenyl acetate, and was isolated, after chromatography on a column of silica gel with solvent *F*, in the form of a syrup that crystallized on trituration with methanol: 128 mg (77%), m.p. 138–139°, $[\alpha]_D$ −34.6° (*c* 1.275, $CHCl_3$); ν_{max}^{KBr} 1740 (OAc),

1700 (C=O), 1660 (NAc), 1600 (C=C), and 1225 cm^{-1} (OAc); λ_{max} 211 and 274 nm (ε_{mM} 13.65 and 3.80).

Anal. Calc. for $C_{20}H_{25}N_5O_8$: C, 51.83; H, 5.44; N, 15.11. Found: C, 51.50; H, 5.69; N, 15.37.

*7-[Methyl 6-O-acetyl-2-(N-acetylacetamido)-2,3,4-trideoxy-α-*D-threo-*hex-2-enopyranosid-4-yl]theophylline* (**15**) was prepared from compound **9** in an analogous way. The crude product was twice rechromatographed on columns of silica gel, eluted first with solvent *C*, and then with solvent *G*. Homogeneous, crystalline product was obtained: 115 mg (52%), which, after recrystallization from ethanol, had m.p. 144–145° and $[\alpha]_D$ +14.7° (*c* 1.05, $CHCl_3$); ν_{max}^{KBr} 1720 (OAc), 1660 (NAc), 1600 (C=C), and 1220 cm^{-1} (OAc); λ_{max} 210 and 274 nm (ε_{mM} 14.30 and 3.70).

Anal. Calc. for $C_{20}H_{25}N_5O_8$: C, 51.83; H, 5.44; N, 15.11. Found: C, 51.80; H, 5.65; N, 15.05.

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