193. 3″-De-*O*-methyl-2″,3″-anhydro-lankamycin, a New Macrolide Antibiotic from *Streptomyces violaceoniger*

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(6. V. 76)

Summary. A further examination of the products from fermentation broths of *Streptomyces violaceoniger* has yielded small quantities of several lankamycin related antibiotics and metabolites. One of the antibiotics has been characterized as 3″-de-*O*-methyl-2″,3″-anhydro-lankamycin. The anhydro sugar of the new lankamycin, 3-methyl-4-*O*-acetyl-2,3,6-trideoxy-α-L-*threo*-hex-2-ene pyranoside was also isolated as the ethyl glycoside from fermentation broths of the same organism.

Lankamycin (**1**), a neutral macrolide with a 14-membered ring aglycone, lankolide, substituted respectively at C(3) and C(5) with sugar moieties 4-*O*-acetyl-arcanose and chalcose, is elaborated by *Streptomyces violaceoniger* [1][1]). Degradative studies by *Keller-Schierlein* & *Roncari* [3] and ^{1}H-NMR. studies by *Egan* & *Martin* [4] established the structure as **1**. More recent ^{1}H-NMR. analysis [5] of degradative products enabled the configurative picture to be completed. During the course of studies on the chemistry of lankamycin we found it necessary to purify additional quantities of the antibiotic from a crude preparation of the original extracted material studied by *Keller-Schierlein* & *Roncari*. This has been particularly rewarding as the crude material yielded, in addition to lankamycin, smaller quantities of several lankamycin related antibiotics and metabolites. The present report describes the isolation and structure of 3″-de-*O*-methyl-2″,3″-anhydrolankamycin (**2**), one of the minor antibio-

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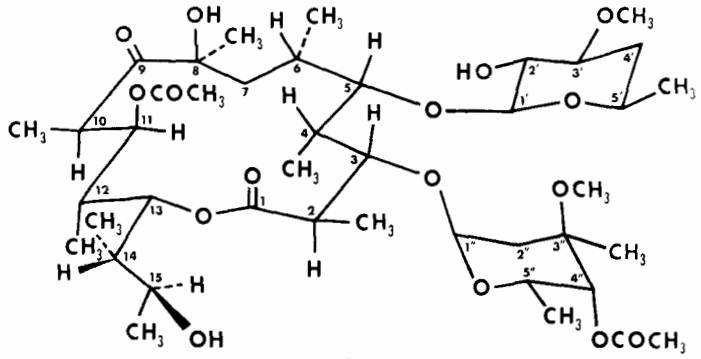
[1]) Japanese investigators have recently isolated lankamycin and 4′-deacetyl-lankamycin from fermentation broths of *S. spinichromogenes* var. *Kujimyceticus* which they termed kujimycin A and B respectively [2]. To minimize confusion alternate names for previously described compounds or their derivatives should be avoided.

tics, and ethyl 3-methyl-4-*O*-acetal-2,3,6-tri-deoxy-α-L-*threo*-hex-2-ene pyranoside (**4**) an ethyl glycoside of the sugar attached at C(3) of **2**.



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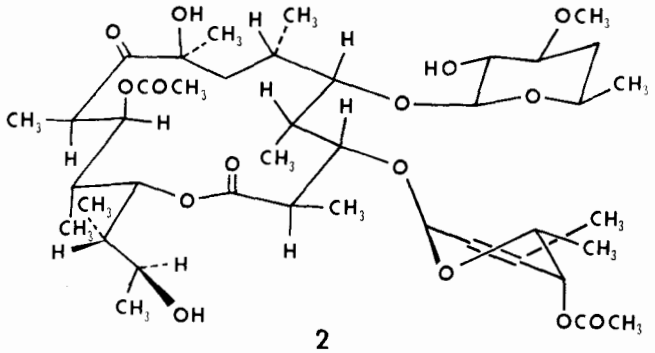

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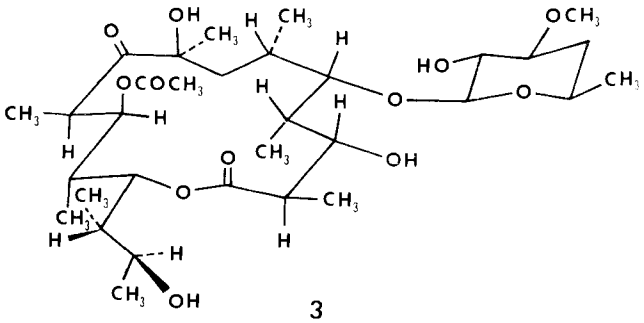
The lankamycin related compounds were isolated from crude lankamycin prepared in 1960 as described previously [1]. Sephadex LH-20 chromatography removed most of the extraneous material from the crude lankamycin preparation. Early eluted fractions yielded lankamycin and related minor antibiotics and later cuts contained lankamycin related sugars and their glycosides. Further chromatography on silica gel or repeated Sephadex LH-20 chromatography was necessary to gain reasonably pure **2**. Purification was difficult and was accomplished with considerable loss so that only a small amount could be obtained pure. Although excellent separations could be achieved using silica gel chromatography, this absorbent was avoided where possible due to extremely poor recovery of chromatographed material.

The first fractions eluted from the initial Sephadex chromatography contained two minor fast moving lankamycin related antibiotics of nearly identical Rf (0.72–0.83). These two compounds were completely resolved on silica gel chromatography. The second eluted, and whose structure is the subject of this paper, 3″-de-*O*-methyl-2″,3″-anhydrolankamycin (**2**), was determined from the observations which follow.

3″-De-*O*-methyl-2″,3″-anhydro-lankamycin (**2**) was recovered as a pale yellow oil which defied all attempts at crystallization. Considerable insight into the structure



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of **2** was obtained by acid catalysed hydrolysis which afforded darcanolide (**3**) characterized by direct comparison of IR. spectra and TLC. behavior with an authentic sample. These data indicated that **2** was closely related to lankamycin as darcanolide is obtained from lankamycin by hydrolysis of the C(3) glycosidic bond with concomitant release of acetyl-acranose [3]. In view of the probable common biogenesis of these metabolites, the undefined portion of **2** was thought to be a sugar

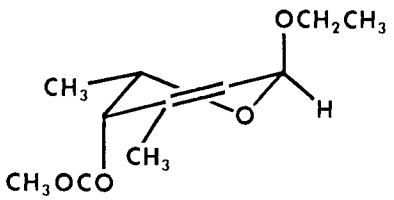

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Table 1. *^1H-NMR. Parameters of Lankamycin* (**1**) *and 3″-De-O-methyl-2″,3″-anhydro-lankamycin* (**2**)

Chemical Shifts [ppm]			Coupling Constants [Hz]		
	1	**2**		**1**	**2**
H–C(2)	2.80	2.63	$J_{2,3}$	5.5	10.0
H–C(3)	3.96	3.76	$J_{3,4}$	2.4	~1
H–C(5)	3.53	3.74	$J_{4,5}$	6.0	~6
H–C(10)	3.15	3.14	$J_{5,6}$	~1	~1
H–C(11)	4.90	5.03	$J_{10,11}$	1.5	~1
H–C(13)	4.87	4.94	$J_{11,12}$	~10	~10
H–C(15)	3.70	~3.7	$J_{12,13}$	~1	~1
H–C(1′)	4.32	4.33	$J_{13,14}$	~9	~9
H–C(1″)	5.04	5.05	$J_{1',2'}$	7.0	7.5
H–C(2″)	–	5.74			
H–C(4″)	4.64	5.02	$J_{1'',2''}$	4.5	3.5
H–C(5″)	4.48	4.22	$J_{4'',5''}$	1.5	2.5
C(3′)–OCH_3	3.44	3.39			
C(3″)–OCH_3	3.30	–			
OAc	2.07	2.06			
	2.12	2.11			
C(3″)–CH_3	–	1.74			

moiety attached to C(3) of darcanolide by glycosidic linkage. These considerations were confirmed by a thorough analysis of the ^{1}H-NMR. spectra (Table 1).

The ^{1}H-NMR. spectra of **2** displayed signals readily recognizable as analogous to those of lankamycin and/or darcanolide. Singlet resonances at 2.06 and 2.11 ppm were attributed to the CH_3-protons of the 11-*O*-acetyl and 4″-*O*-acetyl groups. A characteristic exchangeable singlet at 3.82 ppm was assigned to the C(6) hydroxyl proton. A 3.39 ppm singlet was the only *O*-methyl resonance observed and was attributed to the methoxyl group of chalcose. The anomeric proton resonance of chalcose was observed as a doublet (J = 7.5 Hz) at 4.33 ppm.

Of particular importance were two resonances not observed in previous spectra of lankamycin or related compounds. A broadened methyl resonance with partially resolved triplet fine structure (J = 1.5 Hz) was seen at 1.74 ppm. Spin-decoupling experiments revealed that the unresolved couplings arise from multiplets at 5.74 ppm and 5.05 ppm which were further coupled to each other (J = 3.5 Hz). Consideration of the chemical shifts suggests that the 1.74 ppm methyl resonance and 5.74 ppm multiplet arise from a vinyl methyl and vinyl proton respectively. It is noteworthy that the methoxyl resonance of acetyl-arcanose, previously observed in the spectrum of lankamycin at 3.30 ppm, was absent.

It is apparent that the moiety attached at C(3) must be unsaturated. The site of unsaturation must be 2″,3″ since the 4″-*O*-acetyl chemical shift was similar to that of lankamycin. This assessment was consistent with the following observed ^{1}H-NMR. parameters. The 3″-methyl resonance at 1.75 ppm was coupled to the vicinal 2″-vinyl proton and the allylic 1″-anomeric proton. The coupling between H–C(1″) and H–C(2″) of approximately 3.5 Hz was characteristic of an allylic equatorial proton at C(1″) of a hexopyranosyl derivative vicinally coupled with a vinylic H–C(2″) [6] and indicated an α-L configuration. The resonance of H–C(5″) was found at 4.22 ppm, at slightly higher field than its chemical shift in the spectra of lankamycin (4.48 ppm) or darcanolide (4.47 ppm). Spin decoupling experiments determined the chemical shift of the H–C(4″) resonance at 5.02 ppm ($J_{4''5''}$ = 2.5 Hz).

The structure assignment of **2** was further strengthened by consideration of the chemical ionization mass spectra. Chemical ionization mass spectrometry (CI.-MS.),

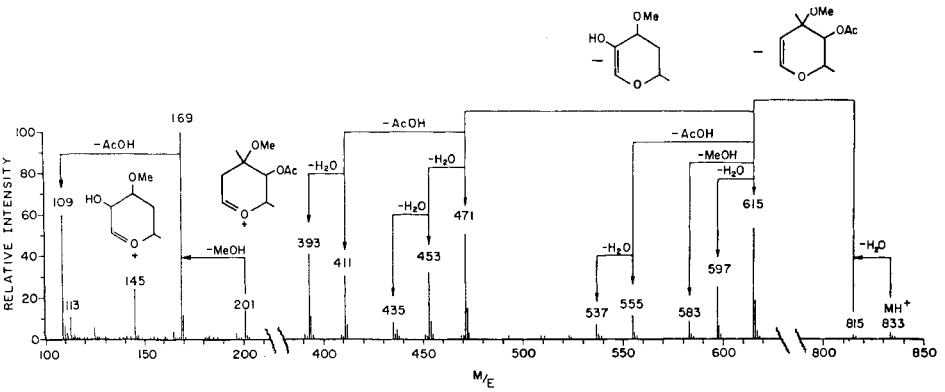


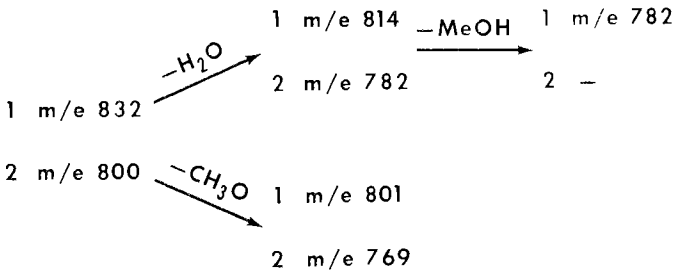
Fig. 1. *Chemical Ionization Mass Spectrum* (CI.-MS.) (iso-butane) *of lankamycin* (**1**)

using isobutane as a reactant gas, has recently been shown to be useful for the characterization of macrolide antibiotics [7]. In particular, protonated molecular ions (MH^+) are normally of high relative intensity and fragmentation generates relatively few abundant ions. Glycosidic bonds are specially susceptible to cleavage, whereas C–C bond cleavages are rarely prominent. The CI.-MS. of lankamycin is reproduced in Fig. 1. The protonated molecular ion gave a small but easily discernible peak at *m/e* 833. All of the prominent fragment ions can be rationalized as resulting from loss of one or more of the oxygen substituents as neutral molecules. The prominent peaks in the low mass portion of the spectrum corresponded to fragment ions of the sugar residues. The oxonium ion of acetyl-arcanose at *m/e* 201 successively loses MeOH and AcOH to give the abundant ions at *m/e* 169 and 109, respectively. Evidence for the second sugar, chalcose, was provided by ions at *m/e* 145 and 113.

Comparison of this spectrum with the isobutane CI-MS. of **2** was quite revealing. The acetyl-arcanose peak at *m/e* 201 was apparently absent with peaks at *m/e* 169 and 109 being the only one attributable to this moiety. The ion of highest mass in the spectrum of **2** occurred at *m/e* 741 and corresponded to the protonated molecular ion minus AcOH. Unfortunately, the protonated molecular ion (*m/e* 801) was not discernible. This is the first example we have seen in which a protonated molecular ion could not be obtained for a macrolide antibiotic using CI-MS. It is quite significant, however, that loss of the anhydro sugar residue occurred in an entirely normal way to give ion *m/e* 615, whose composition was identical with the *m/e* 615 ion derived from lankamycin. The remainder of the spectrum was qualitatively identical with that of lankamycin with the single exception, noted above, of the virtual lack of an ion at *m/e* 201. Thus, the CI.-MS. of **2** is entirely in agreement with the structure proposed on other grounds.

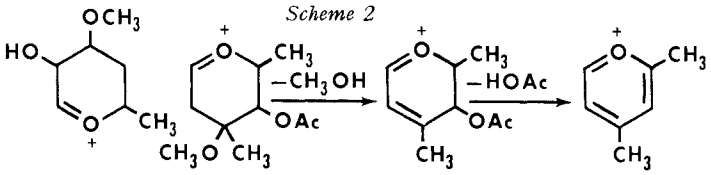
Electron impact mass spectra (EI.-MS.) of compounds in lankamycin series were dominated by sugar fragments and have very weak molecular ions (0.02% for lankamycin), preventing exact mass measurement. Not unexpectedly, **2** did not display a detectable molecular ion (*Scheme 1*). Other ions in the mass spectrum of **2**, including

Scheme 1



the expected series of aglycone ions, were consistent with those observed from **1**. As shown in *Scheme 2* ions **c** and **d** would be expected from either acetylarcanose or the 3″-de-*O*-methyl-2″,3″-anhydro derivative as formulated from ^{1}H-NMR. studies.

Scheme 2



In the course of examining the crude lankamycin preparation we isolated, in addition to the minor lankamycins, several lankamycin related sugars and their derivatives. One of these was the glycoside **4**, ethyl 3-methyl-4-*O*-acetyl-2,3,6-tri-deoxy α-L-*threo*-hex-2-ene pyranoside. The sugar moiety of **4** represented the sugar glycosidically linked to C(3) of **2**. The $CDCl_3$ solution ^{1}H-NMR. spectrum of **4** (Fig. 2,

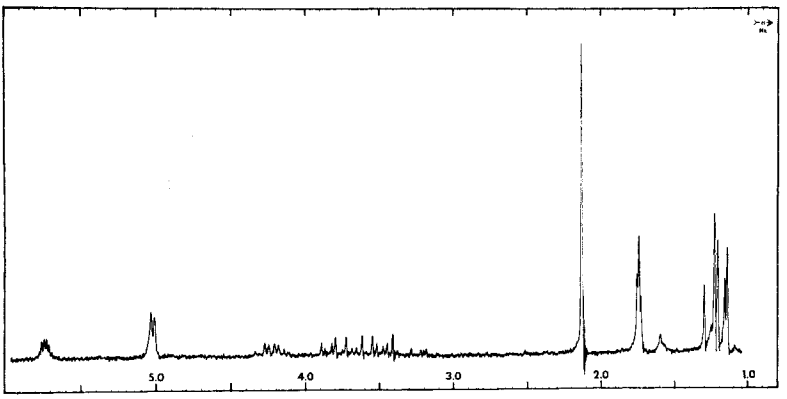
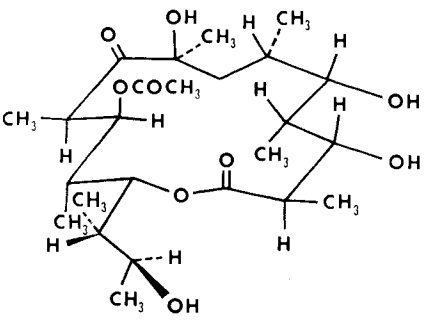


Fig. 2. *^{1}H-NMR. Spectrum of Ethyl 3-Methyl-4-*O*-acetyl-2,3,6-trideoxy-*α*-*L*-*threo*-hex-2-ene Pyranoside* (**4**)

Table 2) confirmed the assignments made from the spectrum of **2.** The chemical shifts and coupling constants collected in Table 2 reveal that the α-L-anomer of **4** was isolated. The high resolution EI.-MS. of **4** failed to show the molecular ion but gave prominent ion (~ 47%) at *m/e* 169 (**a**) due to elimination of $C_2H_5O \cdot$ $(M\text{-}45)^+$. Subsequent loss of AcOH gave rise to an intense ion at *m/e* 109 (100%) consistent



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with the assigned structure from ^{1}H-NMR. analysis. The isolation of glycoside **4** further substantiated the structure assignment of **2** as 3″-de-*O*-methyl-2″,3″-anhydro-lankamycin.

The relatively large amount of darcanolide (**3**) and the occurrence of lankolide (**5**) and arcanose in the lankamycin fermentation extract might be accounted for in part due to the age of the crude preparation (it had been stored since 1960). However, the occurrence of the two macrolide ring components **3** and **5** as natural components would not be unexpected since they are analogous to macrolide biogenetic intermediates erythronolide B and 3-*O*-(α-σ-mycarosyl)erythronolide B identified in the erythromycin biosynthetic pathway and isolated from blocked mutants [8] and commercial high yielding fermentations [9].

Considering the mild conditions used for isolation of the crude lankamycin preparation we were surprised to find α- and β-methyl acetyl-arcanose and ethyl 3-methyl-4-*O*-acetyl-2,3,6-trideoxy-α-L-*threo*-hex-2-ene pyranoside (**4**). To our know-

Table 2. *^{1}H-NMR. Parameters of ethyl 3-methyl-4-*O*-acetyl-2,3,6-trideoxy-α-*L*-threo-hex-2-ene pyranoside* (**4**)

Chemical shifts [ppm]		Coupling constants [Hz]	
H–C(1)	5.02	$J_{1,2}$	3.0
H–C(2)	5.73	$J_{1,3\text{-}CH_3}$	1.5
H–C(4)	5.02	$J_{2,3\text{-}CH_3}$	1.5
H–C(5)	4.23	$J_{4,5}$	2.5
C(1)–CH_2	3.66 [a])	$J_{CH_2\text{-}CH_3}$	7.0
C(1)–CH_3	1.23	$J_{5,5\text{-}CH_3}$	6.5
C(3)–CH_3	1.74		
C(5)–CH_3	1.17		
C(4)–$OCOCH_3$	2.13		

[a]) Center of complex multiplet, ABX_3, $J_{AB} = 9.5$, $\delta = 28$ Hz.

Table 3. *Antibacterial activity of lankamycin* (**1**) *and 3″-de-*O*-methyl-2″,3″-anhydro-lankamycin* (**2**)

Structure	Minimum inhibitory concentration mcg/ml [a])				
	Staphylococcus aureus 9144	*Streptococcus faecalis* 10541	*Bacillus subtilis* 10707	*Sarcina lutea* 9341	*Klebsiella pneumoniae* 10031
1	6.25	50	6.25	0.78	>100
2	>100	>100	>100	25	>100

[a]) Determined by an agar dilution method.

ledge biologically formed methyl and ethyl glycosides of macrolide sugars have not been previously encountered. However, since many glycosyl transferases have very broad specificity and react with a wide variety of acceptors [10] there remains the probability that the isolated glycosides were enzymatically formed.

The previously isolated lankamycins exhibited weak antibacterial activity against certain gram-positive microorganism [1] [2]. 3″-De-*O*-methyl-2″,3″-anhydro-lankamycin (**2**) is nearly inactive showing only minimal activity against easily inhibited strains, *e.g.*, *Sarcina lutea* (Table 3).

The authors gratefully acknowledge Dr. *T. J. Perun*, Abbott Laboratories for his valuable suggestions.

Experimental Part

General remarks. Instrumental methods of analysis have been previously described [11]. TLC. was performed on *Merck* (Darmstadt) pre-coated silica gel 60 F-254 plates using chloroform, 95% ethanol, 10:1 (*v/v*), as the developing solvent. Compounds were visualized by spraying with freshly prepared anisaldehyde reagent (95% ethanol/conc. sulfuric acid/anisaldehyde 10:1:1 (*v/v*)) and heating.

Fractionation of crude lankamycin. A crude lankamycin powder prepared and stored since 1960 was the starting material for isolation of the lankamycin related metabolites of *S. violaceoniger*. In a typical experiment 2.0 g of crude antibiotic was fractionated chromatographically using a Sephadex LH-20 column (3.5 × 90 cm) prepared and eluted with methanol. Fractions were examined by TLC. and where appropriate similar fractions were combined to give the following separations:

Fraction A: Contained two minor lankamycin related antibiotics (67 mg);

Fraction B: Composed of at least three minor compounds, the major portion of the lankamycin (**1**) and 3″-de-*O*-methyl-2″,3″-anhydro-lankamycin (**2**) (745 mg);

Fraction C: Composed of lankamycin and darcanolide (647 mg);

Fraction D: Contained α-L-(4-*O*-acetyl)-arcanose, an unidentified sugar and an unknown lankamycin derivative (51 mg);

Fraction E: Consisted of α- and β-methyl-4-*O*-acetyl-arcanoside (28 mg);

Fraction F: Composed of 4-*O*-acetyl-arcanose and its methyl glycosides and ethyl 3-methyl-4-*O*-acetyl-2,3,6-trideoxy-α-L-*threo*-hex-2-ene pyranoside (**4**) (159 mg)

Fraction G: Composed of pure ethyl 3-methyl-4-*O*-acetyl-2,3,6-trideoxy-α-L-*threo*-hex-2-ene pyranoside (**4**) (23 mg).

Isolation of 3″-de-O-methyl-2″,3″-anhydro-lankamycin (**2**). Fraction B was further fractionated by chromatography on a column (1.8 × 75 cm) of Sephadex LH-20 prepared and eluted with chloroform/hexane 1:1 (*v/v*)[2]. Repeated chromatography on the same system and pooling of homologous fractions containing 3″-de-*O*-methyl-2″,3″-anhydro-lankamycin (**2**) gave 24 mg of pure material which defied crystallization, $[\alpha]_D^{23} = +1°$ (*c* = 1.0, CH_3OH). – IR.: 3590, 3470, 1732, 1230 cm^{-1}. – ^{1}H-NMR.: see Table 1. – EI.-MS.: (*m/e*): 782 (< 0.01%, $M^+ - H_2O$), 802 (< 0.01%, $M^+ - CH_3O$), 471 (3%), 169 (100%), 145 (14%), 109 (93%).

Hydrolysis of 3″-de-O-methyl-2″,3″-anhydro-lankamycin (**2**). *Isolation of darcanolide* (**3**). A solution of 12 mg of 3″-de-*O*-methyl-2″,3″-anhydro-lankamycin (**2**) in 0.5 ml of dioxane was treated with 1.0 ml of 0.1 N H_2SO_4 and the solution heated at 75° for 2 h. The mixture was neutralized with solid $BaCO_3$ and the solid filtered off. Removal of the solvent and subsequent column chromatography on Sephadex LH-20 with methanol afforded a product (5 mg) identical with darcanolide (**3**) in all respects.

Isolation of ethyl 3-methyl-4-O-acetyl-2,3,6-trideoxy-α-L-threo-hex-2-ene pyranoside (**4**). As previously mentioned Fraction G consisted of pure ethyl 3-methyl-4-*O*-acetyl-2,3,6-trideoxy-α-L-*threo*-hex-2-ene pyranoside (**4**). Further material (29 mg) was isolated from Fraction F by upflow column (1.8 × 90 cm) chromatography on Sephadex LH-20 in chloroform. Fractions containing **4** were collected and concentrated to dryness to give additional **2**. – IR.: 1745 and 1600 cm^{-1}. – ^{1}H-NMR.: see Table 2 and Fig. 2. – EI.-MS. (*m/e*): 169 (47%, $M^+ - C_2H_5O$), 109 (100%, $M^+ - C_2H_5O, - HOAc$).

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194. Facteurs stériques influençant la transposition de *Wagner-Meerwein* des carbocations de type pinanyle

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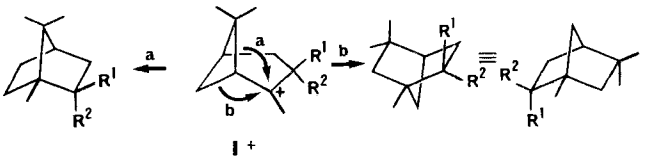
(8. I. 76)

Steric factors influencing *Wagner-Meerwein* rearrangement of pinanyl carbonium ions. – *Summary.* The course of the *Wagner-Meerwein* rearrangement of ions obtained by protonation of substituted α- or β-pinenes is strongly dependent on the configuration of the substituents. The situation is complicated by the possibility of double bond isomerisation (α- → β-pinenes) before rearrangement. Simple rules for predicting the products are given.

Un travail antérieur [1] a montré que le cours des transpositions de *Wagner-Meerwein* des ions carbénium I^+ *(schéma 1)*, obtenus par l'action des acides halohydriques sur des α- et/ou β-pinènes, dans des conditions peu ionisantes, dépend fortement de l'encombrement stérique rencontré dans les transpositions bornylique (a) ou fenchylique (b).

Schéma 1



I $^+$

Ainsi les cyclo-alcènes **1** et **2** (*schéma 2*) donnent des pourcentages différents d'halogénures bornyliques **3** et **4** (45 resp. 95%) et fenchylique **5** (55 %), par action d'acide bromhydrique en solution chloroformique. Or, la paire d'ions (Ia^+X^-, IIa^+X^-) premièrement formée [2] peut conduire au cyclo-alcène à double liaison endocyclique **6** ou **7** après élimination d'un proton, ou bien subir une transposition dont la nature dépendra de la configuration de l'atome de carbone sur lequel est fixé l'atome de brome, ce dernier étant soumis aux encombrements stériques du méthyle-9 et des substituants sur C(3).

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