251. Minor Lankamycin-Related Antibiotics from *Streptomyces violaceoniger*

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Summary

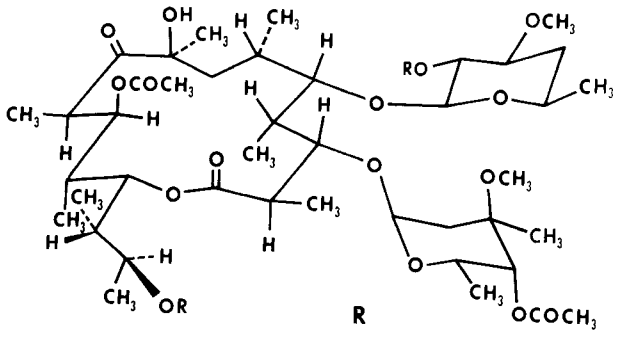
Several previously undescribed metabolites related to lankamycin have been isolated from fermentation broths of the lankamycin-producing organism *Streptomyces violaceoniger*. Two of these metabolites, of weak antibacterical activity, have been characterized as 15-*O*-(*α*-L-4-*O*-acetyl-arcanosyl)-lankamycin and 15-deoxy-15-oxo-lankamycin. The latter antibiotic is prepared in high yield by *Jones* oxidation of lankamycin.

In a previous paper [1] we reported the isolation and structure of a new antibiotic, 3″-de-*O*-methyl-2″,3″-anhydro-lankamycin (**5**) coproduced with lankamycin (**1**) and congeners in submerged fermentations of *Streptomyces violaceoniger*. We indicated that several additional lankamycin-related metabolites were also present in fermentation broths of the same organism. This communication describes the isolation and structure of two of these: 15-deoxy-15-oxo-lankamycin (**2**) and 15-*O*-(*α*-L-4-*O*-acetyl-arcanosyl)-lankamycin (**3**).

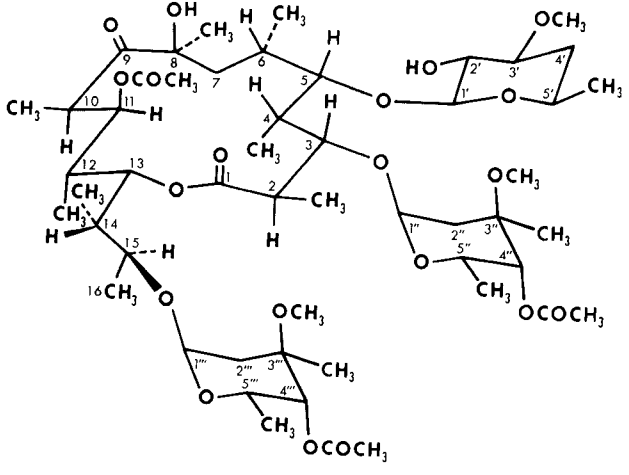
These minor antibiotics were isolated from crude lankamycin prepared in 1960 [2]. A previous report [1] detailed the partial fractionation of this crude material. Rechromatography of Fraction B[1] over Sephadex LH-20 in a chloroform/hexane system gave fractions containing an antibiotic which on TLC. plates gave a characteristic yellow-orange colour when sprayed with anisaldehyde reagent. The antibiotic, now identified as 15-deoxy-15-oxo-lankamycin (**2**) gave an elemental analysis in good agreement with $C_{42}H_{70}O_{16}$, two hydrogen atoms less than the elemental composition of lankamycin.

Comparison of the ^{1}H-NMR. spectrum of **2** with that of lankamycin (**1**) revealed a third acetyl methyl resonance in the 2.0–2.2 ppm region *(Table 1)*. In addition, the highest field methyl doublet, assigned to the C(15)-methyl, and the H–C(15) resonance were both absent. The remainder of the spectrum of **2** was nearly identical with that of lankamycin (**1**).

The ^{13}C-NMR. spectrum *(Table 2)* compared with assignments for lankamycin



	R
1	H
4	$COCH_3$



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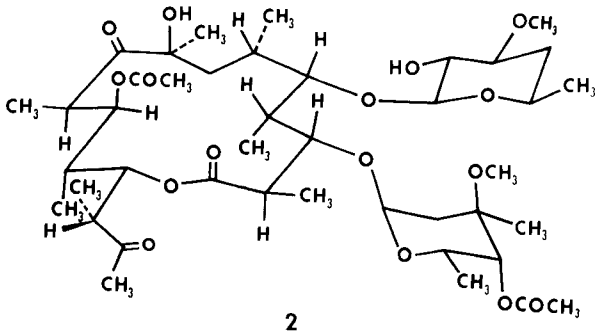

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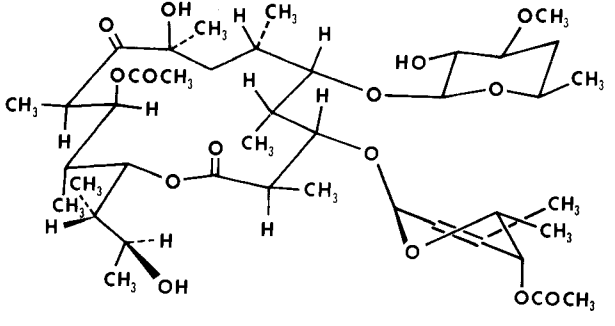

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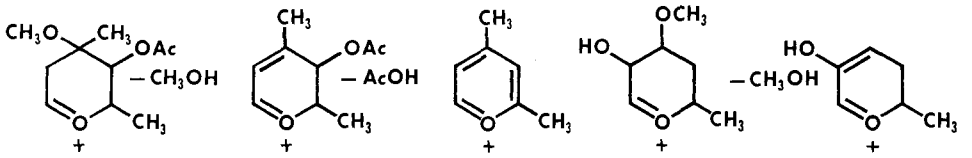
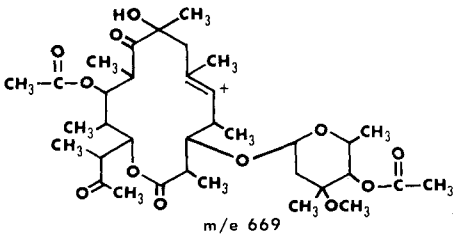
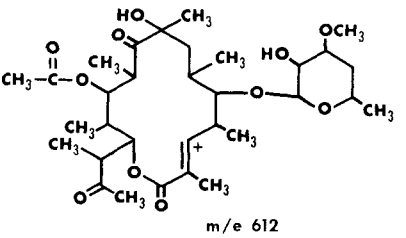
(**1**) [3][1]), displayed an additional ketone carbonyl resonance below 200 ppm, and downfield shifts of C(14) and the two methyl groups at C(14) and C(15).

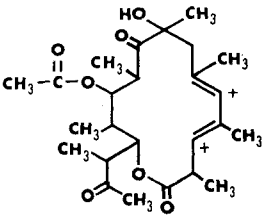
The high resolution electron impact mass spectrum [1] failed to show a molecular ion [1] but indicated prominent ions indicative of 4-*O*-acetyl-arcanose (**a**–**c**) and chalcose (**d**–**e**). The ion **f** ($C_{35}H_{57}O_{12}$, *m/e* 669) arose by elimination of chalcose and ion **g** ($C_{32}H_{52}O_{11}$, *m/e* 612) by loss of arcanose. The aglycone of **2** was represented by ion **h** ($C_{25}H_{41}O_9$, *m/e* 485) derived by loss of both sugar moieties.



Finally, brief treatment of lankamycin (**1**) in acetone with *Jones* reagent gave an almost quantitative yield of 15-deoxy-15-oxo-lankamycin (**2**) identical with the naturally occuring antibiotic. All these facts support the structure of 15-deoxy-15-oxo-lankamycin (**2**).







[1]) Several assignments for lankamycin given in *Table 2* are in disagreement with those tabulated in [3] [4], but are in accord with those given by *Nezmély et al.* [5] which appeared after this work was completed. Our assignments are based on single frequency decoupling experiments using published ^{1}H-NMR. parameters [6] and corroborated in every case by comparison with model compounds. The resonances in the 60–100 ppm region were assigned by ORSFD (off resonance single frequency decoupling) experiments and comparison of lankamycin (**1**) with 2′,15-diacetyl lankamycin (**4**). No specific methyl carbon assignments were made.

Table 1. *^{1}H-NMR. data of lankamycin* (**1**), *15-deoxy-15-oxo-lankamycin* (**2**) *and 15-O-(α-L-4-O-acetyl-arcanosyl)-lankamycin* (**3**)

	Chemical Shifts [ppm]		
	1	**2**	**3**
H–C(2)	2.80	2.82	2.79
H–C(3)	3.96	3.93	3.89
H–C(5)	3.53	3.5	–
H–C(10)	3.15	3.09	–
H–C(11)	4.90	4.83	–
H–C(13)	4.87	5.18	–
H–C(14)	–	2.84	–
H–C(15)	3.70	–	–
H–C(1′)	4.32	4.30	5.03
H–C(1″)	5.04	5.04	–
H–C(2″)	–	–	–
H–C(4″)	4.68	4.69	4.68
H–C(5″)	4.48	4.48	4.46
CH_3O–C(3′)	3.40	3.39	3.42
CH_3O–C(3″)	3.44	3.44	3.27
CH_3O–C(3‴)	–	–	3.24
CH_3–C(3‴)	–	–	–
CH_3–C(8)	1.34	1.31	1.30
CH_3–C(15)	0.85	2.08	0.78
AcO–C(11)	2.07	2.15	2.05
AcO–C(4″)	2.12	2.11	2.11
AcO–C(4‴)	–	–	2.13

As indicated previously [1], Fraction A from the partial purification of crude lankamycin consisted of several components. Repeated chromatography of this fraction on Sephadex LH-20 completely resolved several of the individual components. One of these compounds, 15-*O*-(*α*-L-4-*O*-acetyl-arcanosyl)-lankamycin (**3**), was isolated as an oil.

The ^{1}H-NMR. spectrum of **3** *(Table 1)*, compared with the spectrum of lankamycin (**1**), revealed the presence of additional acetyl and methoxyl singlet resonances. In the spectrum of **3**, the low field region was very complex and in many cases resonance overlap prevented specific assignments. Thus, the ^{1}H-NMR. spectrum was not helpful in determining the origin of the additional resonances.

The structure of **3** was clarified by the high resolution electron impact mass spectrum which was dominated by ions of low mass derived from the sugar moieties. The only sugar fragments observed were those normally seen in lankamycin (**1**). The oxonium ion of acetyl-arcanose at *m/e* 201 (**a**) (56%) successively loses MeOH and AcOH to give ions at *m/e* 169 (**b**) (100%) and *m/e* 109 (**c**) (80%). The presence of chalcose was indicated by ions at *m/e* 145 (**d**) (8%) and *m/e* 113 (**e**) (9%).

Glycosides in the lankamycin series failed to give molecular ions [1]. The peak at highest mass in the spectrum of **3** occurred at *m/e* 972 but was too weak for exact mass determination. Structural features established by ^{1}H-NMR. *(Table 1)* and ^{13}C-NMR. *(Table 2)* suggest that **3** is lankamycin with an additional acetyl-arcanose

Table 2. *^{13}C-NMR. data for lankamycin* (**1**) *and derivatives*

ORSFD Multiplicity	Chemical Shifts			
	1	**2**	**3**	**4**
C(1) *s*	176.5	175.3	175.3	175.0
C(2) *d*	44.9	44.9	44.9	44.5
C(3) *d*	77.6	78.1	78.3	74.6
C(4)[a]) *d*	44.1	44.1	44.1	43.9
C(5) *d*	84.6	84.9	85.0	84.5
C(6)[a]) *d*	34.0	34.4	34.4	34.7
C(7) *t*	39.0	38.9	39.0	38.4
C(8) *s*	80.2	80.2	80.3	80.3
C(9) *s*	214.4	215.8	216.1	214.5
C(10) *d*	38.2	38.0	38.1	37.9
C(11)[b]) *d*	71.1	70.4	70.7	71.1
C(12) *d*	39.0	37.6	38.7	37.4
C(13)[b]) *d*	73.0	72.2	73.5	72.8
C(14) *d*	42.6	49.8	40.0	38.7
C(15) *d*	69.0	210.3	73.8	70.7
C(1′) *d*	102.4	102.6	102.6	100.4
C(2′) *d*	75.5	75.5	75.6	78.5
C(3′) *d*	80.1	80.2	80.3	78.7
C(4′) *d*	37.2	37.2	37.2	37.4
C(5′) *d*	67.3	67.3	67.3	67.1
C(1″) *d*	96.7	97.1	97.1	97.1
C(2″) *t*	30.7	31.0	31.0	30.8
C(3″) *s*	72.6	72.6	72.7	72.7
C(4″) *d*	74.0	73.9	74.0	74.0
C(5″) *d*	62.6	62.9	62.7	62.6
C(1‴) *d*	–	–	96.2	–
C(2‴) *t*	–	–	32.1	–
C(3‴) *s*	–	–	72.7	–
C(4‴) *d*	–	–	74.2	–
C(5‴) *d*	–	–	62.0	–
CH_3O–C(3′) *q*	56.9	56.9	56.9	56.4
CH_3O–C(3″) *q*	49.3	49.3	49.3	49.1
CH_3O–C(3‴) *q*	–	–	49.3	–
CH_3COO–C(11) *s*	170.6	170.6	170.6	170.6
CH_3COO–C(4″) *s*	169.7	169.6	169.4	169.8
CH_3COO–C(4‴) *s*	–	–	170.4	–
CH_3COO–C(2′) *s*	–	–	–	170.2
CH_3COO–C(15) *s*	–	–	–	169.5

[a])[b]) May be interchanged.

moiety attached *via* a glycosidic bond. This would require the molecular formula $C_{52}H_{88}O_{22}$ (mol-wt. 1033.22). Elemental analysis is in agreement with this formula but is not precise enough for definite determination. These observations suggest that the ion at *m/e* 972 resulted from elimination of AcOH from the parent compound.

Ions at *m/e* 771 and *m/e* 827 in the high mass region of the spectrum could be formed by glycosidic bond cleavage of acetyl-arcanose and chalcose respectively. The remaining prominent high mass fragments at *m/e* 940 and *m/e* 954, which must arise by elimination of CH_3OH followed by H_2O from the *m/e* 972 fragment were uninformative.

Inspection of the ^{13}C-NMR. spectrum of **3** *(Table 2)* shows resonances corresponding to ten additional carbon atoms compared with the spectrum of lankamycin (**1**). The mass spectrum of **3** suggests that these additional resonances are most probably due to an additional acetyl-arcanose moiety. The chemical shifts and ORSFD[1]) multiplicities of the additional resonances support the identification of the third sugar moiety as acetyl-arcanose attached by an α-L-glycosidic linkage. The site of attachment was clearly shown to be the C(15) hydroxyl group by the downfield β-shifts exhibited by the C(15) resonances of lankolide and the upfield γ-shift of C(14). The alternate site of attachment at the C(2′)-hydroxyl can be eliminated since the resonances attributed to C(1′), C(2′), and C(3′) are unaffected.

The data allowed assignment of the structure as 15-*O*-(α-L-4-*O*-acetyl-arcanosyl)-lankamycin (**3**).

In an agar dilution assay the minimum inhibitory concentrations of 15-deoxy-15-oxo-lankamycin (**2**) and 15-*O*-(α-L-4-*O*-acetyl-arcanosyl)-lankamycin (**3**) *vs. Staphylococcus aureus* ATCC 6538P were 25 and 50 mcg per ml, respectively. The value for lankamycin (**1**) was 12.5 mcg per ml in the same test.

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Experimental Part

General Remarks. – Instrumental methods of analysis have been previously described [6]. TLC. was performed as described earlier [7]. Fractionation of crude lankamycin was previously reported [1].

Isolation of 15-deoxy-15-oxo-lankamycin (**2**). In a typical isolation a preparation equivalent to Fraction B [1] (736 mg) was chromatographed on a column (3.4×90 cm) of Sephadex LH-20 prepared and eluted with chloroform/hexane 1:1 *(v/v)*. Examination of the fractions by TLC. showed the presence of lankamycin (**2**), 3″-de-*O*-methyl-2″,3″-anhydro-lankamycin (**5**) and a small quantity of material which gave a characteristic yellow-orange colour with anisaldehyde reagent. Fractions containing the latter compound but contaminated with **5** were rechromatographed on a column (1.8×85 cm) of Sephadex LH-20 in the solvent system mentioned above. Slow elution gave fractions containing only the compound which gave the yellow-orange colour with anisaldehyde reagent. Evaporation of the solvents gave 8.63 mg of pure 15-deoxy-15-oxo-lankamycin (**2**) which readily crystallized from ethyl acetate/hexane: m.p. 139–141°; $[\alpha]_D^{19}$ −85.5° (c = 1.0, methanol). – IR.: 3601, 3484, 1735 cm^{-1}.

$C_{42}H_{70}O_{16}$ (831.01) Calc. C 60.70 H 8.49% Found C 60.86 H 8.79%

Isolation of 15-O-(α-L-4-O-acetyl-arcanosyl)-lankamycin (**3**). Fraction A [1] was the starting material for isolation of 15-*O*-(α-L-4-*O*-acetyl-arcanosyl)-lankamycin (**3**). In a typical experiment Fraction A (61.5 mg) was chromatographed on a column (1.8×85 cm) of Sephadex LH-20 prepared and eluted with methanol. Fractions containing the majority of the fastest moving component were collected and concentrated to dryness to give 13.1 mg of crude **3**. Further purification on a column (1.4×75 cm) of Sephadex LH-20 prepared and eluted with chloroform/hexane 1:1 *(v/v)* gave 6.3 mg

of 15-*O*-(α-L-4-*O*-acetyl-arcanosyl)-lankamycin (**3**) as an oil: $[\alpha]_D^{24} - 94.5°$ ($c = 1.03$, methanol). UV. (methanol): λ_{max} 277 (ε 86). – IR.: 3600, 3480, 1740, 1710 cm^{-1}.

$C_{52}H_{88}O_{20}$ (1033.22) Calc. C 60.45 H 8.58% Found C 60.14 H 8.78%

15-Deoxy-15-oxo-lankamycin (**2**) *from lankamycin* (**1**). A stirred solution of lankamycin (**1**) (1.0 g) in 125 ml of acetone cooled in a salt-ice bath was treated for 4 min under N_2 with 0.76 ml of *Jones* reagent [8]. Methanol (2.0 ml) was added, and the reaction mixture poured into 800 ml of 5% aq. $NaHCO_3$. Extraction of the bright green solution with CH_2Cl_2 gave, after drying ($MgSO_4$) and evaporation of the solvent, 1.0 g of colourless foam. Crystallization from ethyl acetate/hexane gave 0.91 g of fine, colourless needles, identical (m.p., ^{1}H-NMR., IR., TLC.) with 15-deoxy-15-oxo-lankamycin (**2**) isolated from fermentation broths.

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