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Structures of Steffimycin and Steffimycin B[1]

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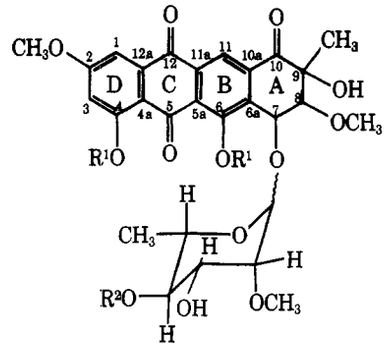
A combination of chemical degradation and spectral studies has established that the structures of steffimycin and steffimycin B are those indicated by structures **1a** and **1b**, respectively.

The discovery of the antibiotic steffimycin (**1a**), produced by *Streptomyces steffisburgensis* and having activity against gram-positive organisms, was reported by Bergy and Reusser[3] some years ago. Subsequently, a description of the isolation of an antibiotic, steffimycin B (**1b**), having very similar physical, chemical, and biological properties, was published.[4] Brodasky and Reusser,[4] on the basis of private communications from Dr. R. C. Kelly, proposed a gross structure for steffimycin. Physical data of various kinds indicated that steffimycin and steffimycin B differed only by the presence of a methyl group in the latter which was absent in the former, and a structure was proposed for steffimycin B. However, the identity of the sugars present in these antibiotics was not published and very limited data were presented. The present paper proposes complete structures (**1a** and **1b**) for these antibiotics, except for stereochemistry in ring A of the linear tetracyclic system, and discusses the data on which these structures are based.

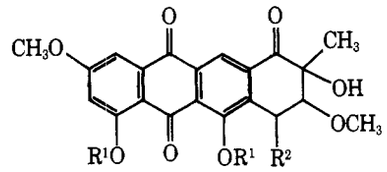
The original publication[3] on steffimycin established that it has a moleclar formula of $C_{28}H_{30}O_{13}$. The ultraviolet spectrum has maxima at 214, 236, 378, and 439 nm with the latter moving to 528 nm in base, which suggests that **1a** has a hydroxyanthraquinone chromophore[5-8] and is related to the anthracycline antibiotics.[9-11] It has been shown[5,6] that such a spectral pattern is present only in hydroxyanthraquinones having two hydroxyl groups α to the quinone carbonyl groups, and that these must be either 1,5 or 1,8. The infrared spectrum has bands at 1672 and 1620 cm^{-1}, which would be those expected for the hydrogen-bonded (1620 cm^{-1}) and nonbonded carbonyls of a 1,8-dihydroxyanthraquinone system.[12] Furthermore, the ^{13}C NMR spectrum of **1a** (Table I) has resonances at δ 179.3 and 189.1 which would arise from such an anthraquinone.[13] Conversion of the phenolic hydroxyls to methoxyls as in **1c** (see below) causes the downfield carbonyl resonance to shift to δ 181.4. In addition, an infrared band at 1710 cm^{-1} indicates a third carbonyl. The ^1H NMR (Me_2SO-d_6) spectrum of **1a** has chemical shifts of δ 6.75, 7.08, and 7.97 arising from aromatic protons present. Signals at δ 1.27 (d, 3 H) and 1.41 (s, 3 H) indicate two CH_3C groups with one being attached to a carbon bearing a proton. Singlets at δ 3.42, 3.44, and 3.90 can be assigned to CH_3O groups. Steffimycin B (**1b**) was found to have a molecular formula of $C_{29}H_{32}O_{13}$ and very similar spectra, except that one more CH_3O was present.[4] The data derived from **1a** and **1b** are so similar to those reported for aranciamycin[14] that it is clear that the three antibiotics are very closely related.

Acidic methanolysis of steffimycin gave rise to two products. One of these was a high-melting orange-red solid designated steffimycinone (**2a**), and the other was a colorless syrup (**4a**) characterized as a diacetate (**4b**), a mono-*p*-nitrobenzoate (**4c**), and a di-*p*-nitrobenzoate (**4d**). Methanolysis of steffimycin B also gave two products. One of these was shown to be **2a** by comparison of physical properties. The second was a second colorless syrup (**4e**), which differed from **4a.** Compound **2a** was shown by analysis and mass spectrometry to have a molecular formula of $C_{21}H_{18}O_9$. Its ultraviolet and infrared spectra were very similar to those of **1a** and were consistent with the assignment of a 1,8-dihydroxyanthraquinone structure to which was attached an aliphatic moiety containing a carbonyl group. The ^{13}C and ^1H NMR spectra indicated that three aromatic protons as well as one of the CH_3C groups and two of the methoxyl groups were present, one of which was attached to an aromatic ring (s, 3 H, δ 3.90) and one to an aliphatic system (s, 3 H, δ 3.48). The resonance arising from the CH_3C was a singlet, indicating the absence of a proton adjacent to the methyl protons. Doublets at δ 3.62 and 5.24 with coupling constants at 3.0 Hz represented 2 H which must be on adjacent carbon atoms. The molecular formula of **2a** accounts for all but a $C_7H_{12}O_4$ moiety of **1a,** which would suggest that **2a** is formed by methanolysis of **1a** to form an aglycone (**2a**) and a sugar (**4a**), which would have a molecular formula of $C_7H_{14}O_5$.

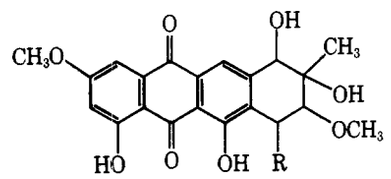
Catalytic reduction of **1a** under low pressure resulted in isolation of a new compound, **2b.** This material was very



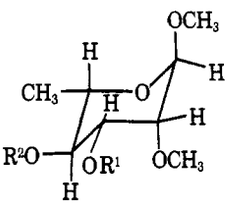
1a, $R^1 = R^2 = H$
b, $R^1 = H$; $R^2 = CH_3$
c, $R^1 = CH_3$; $R^2 = H$



2a, $R^1 = H$; $R^2 = OH$
b, $R^1 = R^2 = H$
c, $R^1 = CH_3$; $R^2 = OH$



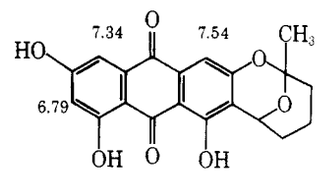
3a, R = OH
b, R = H



4a, $R^1 = R^2 = H$
b, $R^1 = R^2 = CH_3CO$
c, $R^1 = p\text{-}NO_2C_6H_4CO$; $R^2 = H$
d, $R^1 = R^2 = p\text{-}NO_2C_6H_4CO$
e, $R^1 = H$; $R^2 = CH_3$

similar to **2a** in both its ultraviolet and infrared spectra, and thus must contain the chromophore. Analytical data and high-resolution mass spectrometry establish that the molecular formula is $C_{21}H_{18}O_8$. The ^{13}C and ^1H NMR spectra were very similar to those of **2a** in that three aromatic carbon atoms attached to protons, two CH_3O and a CH_3C group, are indicated. However, the doublet in the proton spectrum with a chemical shift of δ 5.24 has disappeared and a new resonance at δ 3.22, arising from 2 H, has appeared. The corresponding change in the ^{13}C NMR spectrum was from δ 62.8 to 26.9. The above data indicate that **2b** (7-deoxysteffimycinone) results from a reductive carbon–oxygen cleavage to remove a sugar moiety attached at a benzylic position.[13d] Methylation of **1a** using dimethyl sulfate and base formed **1c**. The ^1H and ^{13}C NMR spectra of **1c** quite clearly indicated that two new methyl groups attached to oxygen had been introduced. The analysis and mass spectrum were also consistent with formation of a dimethyl ether of **1a**. Similar treatment of **2a** also gave a dimethyl ether (**2c**), as shown by the same sort of evidence. The ^{13}C NMR spectral data derived from **2c** establish that methylation occurred at two phenolic hydroxyl groups.

The ^1H NMR spectra of **1a**, **2a**, and **2b** all indicate the presence of three aromatic protons. Two of these are coupled (J = 2.5 Hz), suggesting that they are 1,3 to each other. The other is a singlet with chemical shifts of δ 7.97–8.02. In addition to the 1,8-dihydroxyanthraquinone system, a methoxyl group attached to an aromatic ring and a $C_6H_{10}O_4$ moiety are present in **2a**. There are then three positions in the anthraquinone which are not substituted and three which are substituted by a combination of CH_3O and OH. This leaves two positions unaccounted for, so the $C_6H_{10}O_4$ moiety must be attached at these positions. The two protons meta to each other can only be in a ring bearing a hydroxyl group meta to a methoxyl group. The other aromatic proton must be α to a carbonyl, as its chemical shift in its ^1H NMR spectrum is too far downfield for that of a β proton.[13d,15] For example, naphthoquinone has chemical shifts of δ 8.07 and 7.77 for α and β protons, respectively. The pattern of aromatic proton resonance is very similar to the similarly substituted averufin[16] (**5**), although



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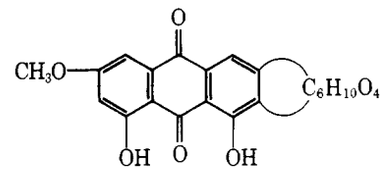


6

differing in absolute values. The partial structure **6** would be a reasonable one for steffimycinone on the basis of these data, although an alternative angular attachment of the six-carbon moiety was possible.

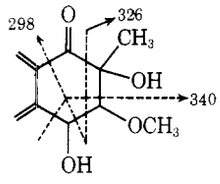
In view of both the ^{13}C and ^1H NMR spectra of **1a** and its degradation products, there must be present in the six-carbon moiety a methoxyl group and a CH_3C on a fully substituted carbon atom. The 1710-cm^{-1} band in the infrared spectrum of **1a** suggests a ketone. This suggestion is confirmed by a chemical shift of δ 198.5 in the ^{13}C NMR spectra of **1a** and one at δ 199.3 in the spectrum of **2a**. The ^{13}C NMR spectra of **2a** and **2c** have three resonances in the range of δ 62.8–87.6. These must arise from aliphatic carbon atoms singly bonded to oxygen, of which one must be that of a CH_3O. The infrared spectrum of **2c** has bands at 3580 and 3420 cm^{-1}, establishing the presence of two hydroxyl groups. The ready conversion of **1a** to **2b** by catalytic hydrogenation indicates an oxygen in a benzylic position, which must be the site of attachment of the sugar. In the formation of **2a**, the sugar is lost, so the substituent at the benzylic position in **2a** must be one of the hydroxyl groups. The ^1H NMR spectrum of **2a** has a resonance appearing as a doublet at δ 5.24, which would be a suitable signal for a proton on a benzylic carbon substituted by oxygen. This proton has a coupling constant of 3 Hz, as does a proton, also appearing as a doublet, at δ 3.62. Consequently, these protons must be on adjacent carbon atoms. In the ^{13}C NMR spectrum of **2a** a carbon bearing a proton resonates at δ 62.8. This must be the benzylic carbon, since it shifts to δ 70.6 in the ^{13}C NMR spectrum of **1a**, in which it is attached in an ether linkage to the sugar moiety. In addition, there is a carbon atom giving a signal in the ^{13}C NMR spectrum of **2a** at δ 87.5. This carbon, as shown by off-resonance decoupling, also has a proton attached. Therefore, it must be the carbon adjacent to the benzylic carbon. Also, because of its resonance at δ 87.5,

Table I. ^{13}C NMR Chemical Shifts[a]

Position	1a Me_2SO-d_6	1b Me_2SO-d_6	1c Me_2SO-d_6	2a Me_2SO-d_6	2b DMF-d_7	2c Me_2SO-d_6	3a Me_2SO-d_6	4a $CDCl_3$	4e $CDCl_3$
C-10	198.5	198.4	200.0	199.3	199.6	199.4	72.9 (69.2)		
C-5	189.1	189.5	181.4	189.6	191.3	181.5	190.2		
C-12	179.3	180.0	179.7	180.0	181.2	179.4	180.9		
C-2	166.5	166.6	163.8	166.5	168.1	163.6	166.2		
C-4	164.6	164.7	161.4	164.6	166.4	161.4	164.5		
C-6	161.3	161.2	160.2	161.3	161.5	160.2	159.9		
C-10a	135.4	135.3	138.4	136.1	137.6	142.0	149.1		
C-11a	134.1	134.6	135.4	135.4	136.7	135.3	134.9		
C-12a	133.3	133.1	135.2	134.6	136.0	133.9	133.3		
C-6a	132.4	132.9	133.7	132.2	132.2	133.6	131.2		
C-5a	117.9	118.4	129.9	117.9	118.1	130.1	113.6		
C-11	115.2	115.3	118.6	115.3	117.0	119.1	118.0		
C-4a	109.5	110.0	117.0	109.9	111.0	116.9	109.9		
C-1	108.0	108.1	104.7	107.9	109.2	104.6	107.7		
C-3	106.1	106.6	104.0	106.4	107.2	102.5	106.4		
C-7	70.6	70.2	72.0	62.8	26.9	63.5	69.2 (72.9)		
C-8	85.5	85.9	86.1	87.5	84.5	87.6	87.6		
C-9	76.2	76.1	76.1	76.3	78.0	76.3	75.2		
CH_3O (C-2)	56.3	56.4	56.3	56.3	57.2	56.2	56.4		
CH_3O (C-8)	59.7	59.7	59.8	59.5	58.8	59.6	60.4		
CH_3O (C-4)			55.8			55.7			
CH_3O (C-6)			62.6			63.1			
CH_3 (C-9)	23.3	23.3	23.4	23.6	22.2	23.8	21.7		
C-1′	100.9	100.7	101.0					97.7	97.5
C-2′	80.5	80.9	80.9					80.5	80.5
C-3′	71.1	71.6	72.2					71.6	71.3
C-4′	72.2	82.3	72.3					73.5	83.8
C-5′	70.3	68.7	70.3					68.0	67.2
CH_3 (C-5′)	17.8	17.8	17.8					17.6	17.9
CH_3O (C-2′)	58.6	59.9	58.7					58.9	58.9
CH_3O (C-4′)		58.5							60.7
CH_3O (C-1′)								54.0	54.7

[a] Assignments were made on the basis of comparisons with other anthracycline antibiotics and compounds derived from them, internal comparisons, values derived from similar compounds in the literature, off-resonance decoupling, and theoretical considerations. Values given are in parts per million downfield from Me_4Si.

it must have the aliphatic methoxyl group as a substituent.[17] In the high-resolution mass spectrum of **2a**, a strong ion (29.4%) is found at 340.05633, indicating a loss of $C_3H_6O_2$. Such a loss can occur only if the aliphatic methoxyl is adjacent to the benzylic carbon, thus making possible loss of the fragment $HOCHCHOCH_3$. These results establish that the second hydroxyl group is attached to a quaternary carbon atom substituted by CH_3, and the resonance in the ^{13}C NMR spectrum of **2a** at δ 76.3 must arise from such a carbon atom. The mass spectrum of **2a** has a base peak at 326 (M − 88) and a very strong peak at 298 (M − 116). These transitions have metastable ions occurring at 257.0 and 214.5; and this information, combined with high-resolution mass measurements on the 326 and 298 ions, establishes that these fragments arise, respectively, by loss of $C_4H_8O_2$ and $C_5H_8O_3$ fragments from the molecular ion. The four-carbon fragment can only be $CH_3OCHC(OH)CH_3$, and the five-carbon fragment then is this plus the ketonic carbonyl as indicated in **7**, which shows the structure of ring A necessitated by these data.



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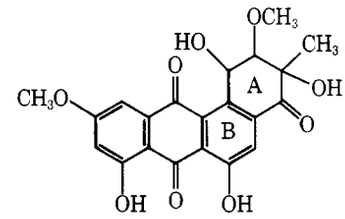
Such a part structure as **7** can be attached to the anthraquinone portion of **2a** in four ways. Both linear attachment as in **2a** and angular attachment as in **8** are possible. Furthermore, each type of attachment can have the orientation of the ketonic carbonyl either as in **2a** or as in **8**. It has already been suggested that the aromatic proton which is on ring B is α to a quinone carbonyl because of its chemical shift (about δ 8) in the ^1H NMR spectra of various compounds discussed. Naphthoquinone was cited as an example supporting this view. Several anthracycline antibiotics have protons α and β to ketonic carbonyls which exhibit similar patterns of chemical shifts in their ^1H NMR spectra. Examples of these are ϵ_1-pyrromycinone, in which an α proton resonates at δ 7.68 while β protons have chemical shifts of δ 7.29,[15c] and α_2-rhodomycinone, which has chemical shifts of δ 8.28 and 7.29 arising from α and β protons, respectively.[15d] However, it might be possible that a proton ortho to a carbonyl as in **8** might resonate at about δ 8, since this is the case with acetophenone. Such considerations would leave as the three possible structures the two orientations of the linear structure and **8**. Reduction of **2a** with a limited amount of sodium borohydride forms steffimycinol (**3a**) by reduction of the ketonic carbonyl group, as would be expected. Spectral evidence (particularly ^{13}C NMR) shows quite clearly that no other change has occurred. In the resultant product (**3a**) there is a



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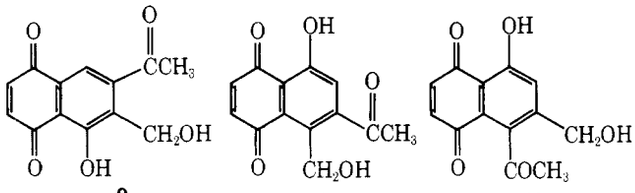
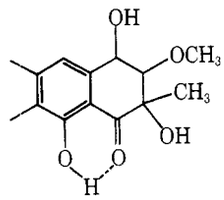
proton which gives rise in the ^1H NMR spectrum to a singlet at δ 7.92 in DMF-d_7. Since the carbonyl group in ring A is no longer present, the downfield position of this proton arises from its position α to the anthraquinone carbonyl, and the linear tetracyclic system must be the correct one. Furthermore, a structure such as 8 would be expected to have both anthraquinone carbonyl groups hydrogen bonded, as is the case in tetrangulol.[18] The ^{13}C NMR spectrum of **2a** provides some evidence bearing on this point. Using values taken from the literature for the chemical shifts in the ^{13}C NMR spectrum of naphthoquinone, and using correction values obtained from Stothers,[17] it can be estimated that the unsubstitued α carbon in **9** would have a chemical shift of δ 117.8 in its ^{13}C NMR spectrum. The corresponding values for the unsubstituted β carbons in **10** and **11** would be δ 119.4 or 124.2 for **10** and δ



122.6 or 127.4 in **11**. The values for the unsubstitued aromatic carbon atom in ring B of **1a** and **2a** are δ 115.2 and 115.3, respectivley. This would be more consistent with the linear tetracyclic system, as in **2a**.

It then remains to establish the orientation of ring A. The frequency of 1710 cm^{-1} found for the ketonic carbonyl in the infrared spectrum of **1a** is higher than would be expected for a carbonyl group attached to an aromatic ring. If the carbonyl group is peri to the hydroxyl group in ring B, as in **12**, hydro-



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gen bonding would be expected, making such a high infrared carbonyl frequency even more surprising. Consequently, this piece of evidence would better fit the **2a** orientation. A number of aglycones contain the C-6, C-7 dihydroxyl arrangement indicated in **2a**. In such cases, the proton at C-7 shows chemical shifts of δ 5.10–5.45 in ^1H NMR spectra.[13d,15c] In steffimycinone, the corresponding resonance is at δ 5.25, which argues strongly for a similar relationship in **2a** and requires that the ketonic carbonyl be at C-10. Bell[19] has shown that metal hydride reduction of phenolic carbonyl compounds leads to reduction to methylene if the carbonyl is ortho or para to the phenolic hydroxyl, but only to a hydroxyl group if the relationship is meta. Reduction of **2a** using an excess of sodium borohydride gave a new compound (**3b**), in which two transformations were evident. In the ^1H NMR spectrum of **3b** the resonance at δ 5.25 had disappeared, showing that the secondary hydroxyl group in ring A was now absent. In addition, the infrared spectrum of **3b** did not have a bond for the ketonic carbonyl. A new singlet at δ 4.33 in the ^1H NMR spectrum of **3b** could only arise by reduction of the ketonic carbonyl to a hydroxyl group. In view of Bell's findings, the only relationship of the substituents in rings A and B which could give such a reduction would be that indicated in **2a**, which must represent the structure of steffimycinone aside from stereochemistry at the asymmetric carbon atoms. At present, very little can be deduced about these configurations. A coupling constant of 3.0 Hz between protons at C-7 and C-8 would suggest that they cannot be diaxial, and therefore the hydroxyl at C-7 and the methoxyl at C-8 cannot both be equatorial.

As previously mentioned, acidic methanolysis of **1a** and **1b** gave, in addition to **2a**, **4a** and **4e**, respectively. The molecular formulas of the two antibiotics, when compared to that of **2a**, indicated that the products, taking into account the addition of CH_3OH, would have molecular formulas of $C_8H_{16}O_5$ (**4a**) and $C_9H_{18}O_5$ (**4b**). Such formulas suggested that these compounds were sugars, as did their ready removal by acidic methanolysis. The ^1H and ^{13}C NMR spectra of **4a** showed quite clearly that it was an eight-carbon compound having a CH_3C and two CH_3O groups, an anomeric carbon and proton, four carbon atoms substituted by oxygen and carrying protons, and suggested a rhamnose configuration. Acetylation formed a diacetate, which was found to be identical with the diacetate of methyl 2-*O*-methyl-α-L-rhamnoside by comparison of its melting point, rotation, and ^1H NMR spectrum with the same properties reported in the literature.[20] The infrared and mass spectra were also consistent with such a structure. Thus, steffimycin must have the structure, aside from stereochemistry, represented by **1a**. Keller-Schierlein et al.[14,20] have proposed that the configuration at C-1 of the sugar in aranciamycin is β on the basis of a ^1H NMR chemical shift at δ 5.49 appearing as a singlet. An almost identical resonance (δ 5.43) occurs in the spectrum of **1a**, but it is not well resolved and appears to be a doublet with a small coupling constant. This may be evidence for an α configuration at C-1 in the sugar in **1a**, as is the case in most anthracyclines.

In view of the structure of **4a** and the differences and similarities observed between **4a** and **4e**, it seemed probable that **4e** was an *O*-methyl analogue of **4a** with methylation having occurred at the oxygen on C-3 or C-4. It was also possible that **4e** was either α- or β-methyl 3,4-di-*O*-methyl-rhamnoside. A comparison of the rotation of **4e** in $CHCl_3$ and CH_3OH with values reported in the literature[21] gave methyl 2,4-di-*O*-methyl-α-L-rhamnoside as the closest match. The ^1H NMR spectrum of **4e** substantiates the identity. Three singlets (δ 3.35, 3.49, and 3.57) show the presence of three methoxyl groups. A doublet at δ 4.71 (J = 1.5 Hz) represents the anomeric hydrogen, which is coupled to a proton at C-2 (δ 3.45, J = 1.5 and 3.7 Hz) which resonates as a doublet of doublets. In such case, the H-1, H-2 relationship is ee or ea, as is the relationship of H-2 to H-3 (δ 3.80). The latter gives rise to six lines with J values of 3.7 (H-2, H-3), 9 (H-3, H-4), and 8.8 Hz (H-3 and OH). The hydroxyl proton has a chemical shift of δ 2.74 (d, J = 8.8 Hz). The proton on C-4 resonates as a triplet at δ 2.97 (J = 8.3 and 9 Hz), with the smaller coupling constant arising from coupling with a proton on C-5 appearing as a multiplet at δ 3.55. These coupling constants establish that the H-3, H-4 protons are aa, as are H-4 and H-5. The proton on C-5 is coupled with the CH_3C proton, which shows as a doublet (δ 1.29, J = 6.4 Hz). This spectrum establishes that **4e** is methyl 2,4-di-*O*-methylrhamnoside and the rotation establishes that it is α-L. The structure of steffimycin B can then be depicted as **1b**, although again stereochemistry is not completely established, but must be the same as in **1a**.

Experimental Section

Steffimycinone (2a). (a) From Steffimycin (1a). A solution of 5 g (8.7 mmol) of **1a** in 500 mL of 1 N methanolic hydrochloric acid was boiled under reflux for 128 h. The reaction mixture was refrigerated and filtered, yield 2.84 g. A second crop of 0.5 g was obtained from the filtrate. These fractions were combined and recrystallized from CH_3OH to give 2.45 g, mp 248–250 °C, and a second crop of 0.74 g, mp 245–249 °C, yield 87%. Two further recrystallizations from CH_3OH gave orange prisms: mp 250–251.5 °C; R_f 0.31 (SiO_2; CH_2Cl_2–CH_3OH, 95:5); UV (EtOH) λ_{max} 213 nm (ϵ 26 300), 236 (ϵ 28 180), 257 sh (ϵ 20 420), 279 (ϵ 20 430), 439 (ϵ 14 130); IR (Nujol) 3500, 3070, 1710, 1675, 1625, 1600, 1560, 1315, 1250, 1200, 1160, 1105,

1035, 960, 755 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.43 (s, 3 H, CH_3C), 3.48 (s, 3 H, CH_3O), 3.62 (d, 1 H, J = 3.0 Hz, H-8), 3.90 (s, 3H, CH_3O), 5.24 (d, 1 H, J = 3.0 Hz, H-7), 6.75 (d, 1 H, J = 2.5 Hz, H-3), 7.10 (d, 1 H, J = 2.5 Hz, H-1), 8.00 (s, 1 H, H-11); mass spectrum m/e 414.09538 (15.7; calcd for $C_{21}H_{18}O_9$, 414.09508), 340.05633 (− $CH_3OCHCHOH$, 29.4; calcd for $C_{18}H_{12}O_7$, 340.05830), 326.04236 (− $CH_3OCHC(OH)$-CH_3, 97.2; calcd for $C_{17}H_{10}O_7$, 326.04265), 298.04748 (− CH_3O-$CHC(OH)CH_3CO$, 36.4; calcd for $C_{16}H_{10}O_6$, 298.04773).

Anal. Calcd for $C_{21}H_{18}O_9$: C, 60.87; H, 4.38. Found: C, 60.14; H, 4.77.

(b) From Steffimycin B (1b). 1b (5 g) was treated with acidic methanol as above, except that heating was continued for 48 h only. The first fraction weighed 2.2 g. A second fraction of 1.6 g was obtained by concentration and refrigeration of the filtrate from the first fraction. These fractions were combined and chromatographed on 360 g of silica gel using cyclohexane–ethyl acetate–ethanol (6:3:1) as the eluting system and collecting 486 10-mL fractions. Fractions 290–486 were combined and concentrated under reduced pressure. The residue was recrystallized from acetone, yield 1.07 g, mp 253–256 °C. The product had the same R_f (0.38) on TLC (SiO_2, cyclohexane–ethyl acetate–ethanol, 5:3:2) as did **2a**. Its IR spectrum was the same as that of **2a**, and a mixture melting point was not lowered.

7-Deoxysteffimycinone (2b). 1a (1 g) was dissolved in CH_3OH, and 300 mg of 10% Pd/C was added. The mixture was shaken under hydrogen at an initial pressure of 45 psi for 93 h. The catalyst was removed by filtration, and the filtrate was evaporated to dryness under reduced pressure. The residue was pratitioned between CH_2Cl_2 and H_2O, and the two-phase system was filtered. The water layer was removed, and the organic layer was washed with water, a solution containing an excess of $FeCl_3$ in 1 N HCl, and again with water. The organic phase was removed and concentrated to dryness under reduced pressure. The residue was chromatographed on 100 g of silica gel using CH_2Cl_2–CH_3OH (97:3) for elution, which was continued until the material having R_f 0.40 (SiO_2; CH_2Cl_2–CH_3OH, 97:3) had been eluted. The fractions containing this material were combined and evaporated to dryness under reduced pressure, weight 160 mg. Recrystallization from CH_3OH gave 85 mg, mp 191–194 °C. Two recrystallizations from EtOH gave: mp 191.5–194 °C; UV (EtOH) λ_{max} 213 nm (ϵ 25 700), 236 (ϵ 27 400), 258 sh (ϵ 19 520), 274 sh (ϵ 21 400), 283 (ϵ 23 450), 458 (ϵ 14 800); UV (0.01 N methanolic KOH) λ_{max} 230 sh nm (ϵ 25 650), 268 (ϵ 23 700), 514 (−11 670); IR (Nujol) 3500, 1705, 1675, 1620, 1605 sh, 1560, 1305, 1240, 1160, 1100, 965, 755 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.33 (s, 3 H, CH_3C), 3.22 (m, 2 H, CH_2), 3.37 (s, 3 H, CH_3O), 3.77 (m, 1 H, H-8), 3.90 (s, 3 H, CH_3O), 6.74 (d, 1 H, J = 2.5 Hz, H-3), 7.17 (d, 1 H, J = 2.5 Hz, H-1), 8.02 (s, 1 H, H-7); mass spectrum m/e 398.09730 (calcd for $C_{21}H_{18}O_8$, 398.1002), 324 (98.5), 323 (100), 310 (9.8), 295 (39.8), 282 (20.1).

Anal. Calcd for $C_{21}H_{18}O_8$: C, 63.31; H, 4.55. Found: C, 63.52; H, 4.57.

4,6-Di-*O*-methylsteffimycin (1c). 1a (1g, 1.67 mmol) was dissolved in 100 mL of acetone, and the air above the solution was displaced with N_2. K_2CO_3 (1 g) was added, followed by 650 mg (4.7 mmol) of $(CH_3)_2SO_4$, and the mixture was heated under reflux for 20 h. Water was added, and the reaction mixture was stirred for 45 min. The acetone was removed by evaporation under reduced pressure. The residue was mixed with CH_2Cl_2, and the mixture was extracted with 5% NaOH solution. The CH_2Cl_2 solution was washed with a saturated ammonium chloride solution and dried ($MgSO_4$). After filtration, the solution was evaporated to dryness under reduced pressure, leaving a residue which was crystallized from CH_3OH. The product obtained was chromatographed on 100 g of silica gel using CH_2Cl_2–CH_3OH (95:5). The fractions containing the first color maximum off the column were combined and evaporated to dryness under reduced pressure. The residue (500 mg) was recrystallized three times from CH_3OH: yield 307 mg; mp 220–224 °C; R_f 0.39 (SiO_2; $CHCl_3$–CH_3OH, 9:1); UV (EtOH) λ_{max} 248 nm (ϵ 25 890), 283 sh (ϵ 16 860), 396 (ϵ 5240); IR (Nujol) 3635, 3570, 3510, 3400, 1705, 1670, 1650, 1590, 1555, 1370, 1340, 1320, 1285, 1275, 1235, 1090, 1050, 1025, 980, 950, 910, 840, 815, 740 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.30 (d, 3 H, J = 6.0 Hz, CH_3CH), 1.43 (s, 3 H, CH_3C), 3.35 (s, 3 H, CH_3O), 3.53 (s, 3 H, CH_3O), 3.68 (d, 1 H, J = 2.2 Hz, H-8), 3.93 (s, 6 H, CH_3O), 3.98 (s, 3 H, CH_3O), 4.68 (d, 1 H, OH), 4.90 (d, 1 H, OH), 5.15 (d, 1 H, J = 2.2 Hz, H-7), 5.42 (d, 1 H, J = 2.2 Hz, anomeric), 5.55 (s, 1 H, OH), 6.84 (d, 1 H, J = 2.5 Hz, H-3), 7.14 (d, 1 H, J = 2.5 Hz, H-1), 8.22 (s, 1 H, H-11); mass spectrum m/e 602 (M^+, 4.5), 472 (9.0), 442 (21.6), 426 (89.1), 383 (100), 352 (82.9).

Anal. Calcd for $C_{30}H_{34}O_{13}$: C, 59.79; H, 5.69. Found: C 59.84; H 5.93.

4,6-Di-*O*-methylsteffimycinone (2c). 2a (100 g, which also contained considerable **1a**) was dissolved in 3.5 mL of acetone, and 77.0 g of K_2CO_3 and 57 mL of $(CH_3)_2SO_4$ were added. The mixture was stirred and heated under reflux under N_2 for 21.5 h. After the reaction mixture had cooled to room temperature it was filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2 containing a small amount of CH_3OH and washed with three portions of 5% NaOH solution. The CH_2Cl_2 solution was washed with H_2O and dried (Na_2SO_4). Filtration and evaporation under reduced pressure gave 105 g. This material was chromatographed in two portions, 34 and 71 g. The smaller portion was chromatographed on 2.4 kg of silica gel, eluting with 10 L of CH_2Cl_2–CH_3OH (98:2), 10 L (97:3), and 4 L (96:4). The second fraction removed from the column (as indicated by TLC) was isolated by evaporation under reduced pressure, yield 17.5 g. The larger fraction was purified similarly to give 36.0 g. These fractions were combined and recrystallized from CH_2Cl_2–CH_3OH, yield 42.5 g, mp 232.5–234.5 °C. A small sample was recrystallized from acetone for analysis: R_f 0.32 (SiO_2; $CHCl_3$–CH_3OH, 95:5), 0.46 (SiO_2; $CH_3COOC_2H_5$–C_2H_5OH–H_2O, 92:5:3); UV (EtOH) λ_{max} 248 nm (ϵ 25 640), 283 sh (ϵ 18 560), 395 (ϵ 10 060); IR (Nujol) 3470, 3420, 1705, 1675, 1660, 1590, 1555, 1370, 1340, 1320, 1285, 1250, 1195, 1170, 1150, 1095, 1040, 1025, 975, 955, 905, 860, 835, 740 cm^{-1}; ^1H NMR (DMF-d_7) δ 1.43 (s, 3 H, CH_3C), 3.59 (s, 3 H, CH_3O), 3.77 (d, 1 H, J = 2.5 Hz, H-8), 4.09 (s, 6 H, $2CH_3O$), 4.09 (s, 3 H, CH_3O), 5.44 (d, J = 2.5 Hz, H-7), 7.01 (d, 1 H, J = 2.5 Hz, H-3), 7.26 (d, 1 H, J = 2.5 Hz, H-1), 8.44 (s, 1 H, H-11); mass spectrum m/e 442.1267 (calcd for $C_{23}H_{22}O_9$, 442.1264).

Anal. Calcd for $C_{23}H_{22}O_9$: C, 62.44; H, 5.02. Found: C, 62.28; H, 5.14.

Steffimycinol (3a). 2a (16 g) was reduced in four equal batches as follows: a solution of 4.0 g (9.7 mmol) of **2a** in 200 mL of 0.2 N NaOH solution was stirred while adding dropwise over 0.5 h a solution of 280 mg (7.6 mmol) of $NaBH_4$ in 40 mL of 0.2 N NaOH solution. Stirring was continued for another 0.5 h, followed by addition of 50 mL of 2 N HCl. The resulting mixture was extracted with one 200-mL portion and two 100-mL portions of EtOAc. The extracts were combined, dried ($MgSO_4$), filtered, and evaporated to dryness under reduced pressure. Sixteen grams of **2a** gave 13.2 g of residue. This material (10.8 g) was deposited from solution on 37 g of silica gel, which was added to the top of a column containing 1080 g of silica gel packed in $CHCl_3$–CH_3OH (97:3). Elution was done with the same solvent system until those fractions containing **3a**, as determined by TLC (R_f 0.18; SiO_2; $CHCl_3$–CH_3OH, 95:5), were eluted. Those fractions containing pure **3a**, also determined by TLC, were combined and evaporated to dryness under reduced pressure: yield 3.7 g (23%); mp 230 °C dec; UV (EtOH) λ_{max} 227 nm (ϵ 35 400), 269.5 (ϵ 21 250), 285 sh (ϵ 17 150), 435 (ϵ 13 200); IR (Nujol) 3600, 3560, 3300, 1675, 1605, 1570, 1565, 1395, 1300, 1275, 1255, 1215, 1160, 1100 cm^{-1}; ^1H NMR (Me_2SO-d_6–D_2O) δ 1.38 (s, 3 H, CH_3C) 3.32 (d, 1 H, J = 6.0 Hz, H-8), 3.63 (s, 3 H, CH_3O), 3.83 (s, 3 H, CH_3O), 4.42 (s, 1 H, H-10), 4.95 (d, 1 H, J = 6.0 Hz, H-7), 6.65 (d, 1 H, J = 3.0 Hz, H-3), 7.00 (d, 1 H, J = 3.0 Hz, H-1), 7.80 (s, 1 H, H-11); mass spectrum m/e 416 (M^+, 1.3), 398 (14.9), 328 (100), 310 (90.2), 299 (22.6), 282 (21.8).

Anal. Calcd for $C_{21}H_{20}O_9$: C, 60.57; H, 4.85. Found: C, 60.55; H, 4.97.

7-Deoxysteffimycinol (3b). A solution of 2.0 g (4.8 mmol) of **2a** in 100 mL of 0.2 N NaOH solution was stirred while adding dropwise 920 mg (24.2 mmol) of $NaBH_4$ dissolved in 46 mL of 0.2 N NaOH solution. Stirring was continued 1 h after addition was completed. EtOAc (150 mL) and 35 mL of 2 N HCl were added. The organic layer was removed, and the aqueous layer was extracted with two 100-mL portions of ethyl acetate. The organic layers were combined, dried ($MgSO_4$), and evaporated to dryness under reduced pressure, weight 1.53 g. Material prepared in this way (2.2 g) was recrystallized twice from $CHCl_3$–CH_3OH (97:3) by dissolving in ~900 mL and concentrating: yield, 1.12 g; mp 274–276 °C; R_f 0.44 (SiO_2, $CHCl_3$–CH_3OH; 95:5); UV (EtOH) λ_{max} 226 nm (ϵ 34 000), 249 sh (ϵ 17 550), 273 (ϵ 24 500), 435 (ϵ 13 500); IR (Nujol) 3490, 1660, 1625, 1610, 1560, 1390, 1315, 1300, 1275, 1240, 1210, 1190, 1095, 970, 805, 785, 760 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.37 (s, 3 H, CH_3C), 3.33 (m, 5 H, H-7, H-8, OH), 3.44 (s, 3 H, CH_3O), 392 (s, 3 H, CH_3O), 4.33 (s, 1 H, H-10), 6.85 (d, 1 H, J = 2.6 Hz, H-3), 7.17 (d, 1 H, J = 2.6 Hz, H-1), 7.87 (s, 1 H, H-11); mass spectrum m/e 400.1172 (calcd for $C_{21}H_{10}O_8$, 400.1158), 350 (32.9) 339 (49.7), 325 (45.7), 312 (100), 284 (70.9).

Anal. Calcd for $C_{21}H_{20}O_8$: C, 63.00; H, 5.04. Found: C, 62.51; H, 4.99.

Methyl 2-O-Methyl-α-L-rhamnoside (4a). The filtrate after crystallization of **2a** derived from 10 g of **1a** was mixed with 150 mL of pyridine, and 1 L of H_2O was added. The solution was extracted repeatedly with 200-mL portions of CH_2Cl_2 until the red color was removed. The aqueous solution was stirred overnight with 300 mL of Dowex 2 (OH^-). The resin was removed by filtration, and the fil-

trate was lyophilized. The residue was dissolved in 200 mL of $CHCl_3$, and the insoluble material was removed by filtration. Evaporation of the filtrate under reduced pressure gave 2.66 g (80%) of syrupy residue, homogeneous by TLC on SiO_2 (R_f 0.50; cyclohexane–ethyl acetate–EtOH, 5:3:2): ^1H NMR ($CDCl_3$) δ 1.32 (d, 3 H, J = 6.0 Hz, CH_3C), 3.43 (s, 3 H, CH_3O), 3.55 (s, 3 H, CH_3O), 3.2–4.0 (m, 4 H, CHO), 4.83 (d, 1 H, J = 1.0 Hz, H-1).

Methyl 2-*O*-Methyl-3,4-di-*O*-acetyl-α-L-rhamnoside (4b). **4a** (1 g) was converted to its diacetate by the standard acetic anhydride–pyridine procedure. The product was a syrup, weight 0.88 g. This material was chromatographed on 88 g of silica gel, eluting with $CHCl_3$–$CH_3COOC_2H_5$ (95:5) and collecting 128 10-mL fractions. As a result of weight analysis fractions 40–60 were combined and evaporated to dryness under reduced pressure, weight 0.73 g. Crystallization from ether–Skellysolve B gave 0.27 g, mp 68–71 °C. The crystalline material was sublimed under a pressure of 0.5 mm and a bath temperature of 60–62 °C: yield 0.24 g; mp 70–72 °C (lit.[20] 70–71 °C); R_f 0.45 (SiO_2; $CHCl_3$–$CH_3COOC_2H_5$, 9:1); $[\alpha]_D$ −71° (*c* 2, CH_3OH) (lit.[20] −69°); IR (Nujol) 1740, 1235, 1220, 1150, 1170, 1140, 1125, 1105, 1070, 1050, 975, 965, 930, 915, 910, 885, 820 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22 (d, 3 H, CH_3C), 2.06 and 2.10 (2s, 6 H, CH_3CO), 3.42 (s, 3 H, CH_3O), 3.50 (s, 3 H, CH_3O), 3.63 (d of d, 1 H, J = 2.0 and 3.2 Hz, H-2), 3.80 (m, 1 H, H-5), 4.73 (d, 1 H, J = 2.0 Hz, H-1), 5.10–5.30 (m, 2 H, H-3 and H-4); mass spectrum *m/e* 245 (M − CH_3O).

Anal. Calcd for $C_{12}H_{20}O_7$: C, 52.16; H, 7.30. Found: C, 52.42; H, 7.23.

Methyl 2-*O*-Methyl-3-*O*-(*p*-nitrobenzoyl)-α-L-rhamnoside (4c) and Methyl 2-*O*-Methyl-3,4-di-*O*-(*p*-nitrobenzoyl)-α-L-rhamnoside (4d). A solution of 209 mg of **4a** and 500 mg of *p*-nitrobenzoyl chloride in 10 mL of pyridine was allowed to stand at room temperature for 72 h. Water (1 ml) was added and the solution was allowed to stand for 0.5 h, after which it was poured into CH_2Cl_2. The pyridine was removed by thorough washing with 1 N HCl. This was followed by washing with 1 N $NaHCO_3$ solution and water. The CH_2Cl_2 solution was dried ($MgSO_4$), filtered, and evaporated to dryness, leaving an oily residue (375 mg). The product was chromatographed on 40 g of silica gel using gradient elution with benzene–ether (98:2 to 8:2). The eluate was analyzed by TLC on SiO_2 (benzene–ether, 8:2), combining the fractions containing a faster moving material (R_f 0.64) and the fractions containing a slower moving material (R_f 0.28). Evaporation of the R_f 0.64 fractions gave **4d** (72 mg) as indicated by its ^1H NMR: ($CDCl_3$) δ 1.33 (d, 3 H, J = 6.5 Hz, CH_3C), 3.50 (s, 3 H, CH_3O), 3.52 (s, 3 H, CH_3O), 3.87 (m, 1 H, H-2), 3.9–4.3 (m, 1 H, H-5), 4.87 (d, 1 H, J = 2.0 Hz, H-1), 5.62 (m, 2 H, H-3 and H-4), 8.21 (s, 8 H, aromatic).

Evaporation of the R_f 0.28 fractions gave **4c** (233 mg), which was crystallized from benzene–ether and from ether–methylene chloride: mp 139–139.5 °C; IR (Nujol) 3470, 1725, 1605, 1525, 1490, 1345, 1275, 1185, 1120, 1115, 1100, 1050, 1040, 885, 835, 725, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34 (d, 3 H, J = 5.8 Hz, CH_3C), 3.37 (s, 3 H, CH_3O), 3.42 (s, 3 H, CH_3O), 3.68 (q, 1 H, J = 2.0 and 3.2 Hz, H-2), ~3.75 (m, 1 H, H-2 or H-4), 3.80 (m, 1 H, H-2 or H-4), 4.73 (d, 1 H, J = 2.0 Hz, H-1), 5.27 (q, 1 H, J = 9.5 and 3.2 Hz, H-3), 8.22 (m, 4 H, aromatic).

Anal. Calcd for $C_{15}H_{19}NO_8$: C, 52.78; H, 5.61. Found: C, 52.77; H, 5.21.

Methyl 2,4-Di-*O*-methyl-α-L-rhamnoside (4e). The filtrate from crystallization of **2a** after methanolysis of 5.0 g of **1b** was evaporated under reduced pressure until the CH_3OH was removed. The residue was mixed with a solution of 75 mL of pyridine in 500 mL of water, and the resulting solution was stirred overnight with 125 mL of Dowex 2 (OH^-). The resin was removed by filtration, and the filtrate was concentrated to a syrup by distillation under 25–30 mm at 45 °C. Water (50 ml) was added to the residue, and the solution was adjusted to pH 9.0 with 1.0 N NaOH solution. The basic solution was extracted with three 50-mL portions of $CHCl_3$. The combined extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure, leaving a mobile liquid, weight 1.01 g, homogeneous by TLC (R_f 0.60; SiO_2; cyclohexane–ethyl acetate–ethanol, 5:3:2). The liquid was chromatographed on 50 g of silica gel, eluting with Skellysolve B–acetone (4:1) until 92 5-mL fractions were collected. Fractions 40–62 (weight maximum) were combined and evaporated under reduced pressure to give a residue weighing 0.93 g. Distillation gave 0.30 g of colorless liquid: bp 82 °C (0.3 mm); $[\alpha]_D$ −67.7° (*c* 2, CH_3OH) (lit.[21a] −66.6°); $[\alpha]_D$ −56.0° (*c* 2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.29 (s, 3 H, J = 6.4 Hz, CH_3C), 3.35 (s, 3 H, CH_3O), 3.49 (s, 3 H, CH_3O), 3.57 (s, 3 H, CH_3O), 2.74 (d, 1 H, J = 8.8 Hz, OH), 2.97 (t, 1 H, J = 9.0 and 8.3 Hz, H-4), 3.45 (d of d, 1 H, J = 3.7 and 1.5 Hz, H-2), 3.55 (m, 1 H, H-5), 3.80 (m, 1 H, J 9.0, 8.8, and 3.7 Hz, H-3), 4.71 (d, 1 H, J = 1.5 Hz, H-1); mass spectrum *m/e* 175.0978 (M − CH_3O) (calcd for $C_8H_{15}O_4$, 175.0970).

Anal. Calcd for $C_9H_{18}O_5$: C, 52.40; H, 8.80. Found: C, 51.90; H, 9.87.

Registry No.—**1a**, 11033-34-4; **1b**, 54526-94-2; **1c**, 63493-71-0; **2a**, 57847-74-2; **2b**, 57847-75-3; **2c**, 63493-72-1; **3a**, 63493-73-2; **3b**, 63493-74-3; **4a**, 59013-63-7; **4b**, 63527-42-4; **4c**, 63493-75-4; **4d**, 63493-76-5; **4e**, 35939-75-4; *p*-nitrobenzoyl chloride, 122-04-3.

References and Notes

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