Synthesis of Oxazinomycin (Minimycin)[1]

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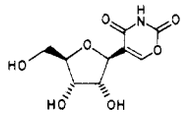
The C-nucleoside antibiotic oxazinomycin (1) has been synthesized, starting with the 2',3'-*O*-isopropylidene-5'-*O*-trityl-D-ribofuranosylacetonitriles **3** and **4**. Formylation of **3** and **4** with bis(dimethylamino)-*tert*-butoxymethane afforded the 3-dimethylamino-2-(2',3'-*O*-isopropylidene-5'-*O*-trityl-D-ribosyl)acrylonitriles **5** and **6**, which by reaction with hydroxylamine were converted to the 5-amino-4-(2',3'-*O*-isopropylidene-5'-*O*-trityl-D-ribosyl)isoxazoles **8** and **9**. Hydrogenation of **8** and **9** gave the 3-amino-2-(2',3'-*O*-isopropylidene-5'-*O*-trityl-D-ribosyl)acrylamides **10** and **11**. These were subjected to hydrolysis and subsequent reaction with *N,N'*-carbonyldiimidazole to furnish the 5-(2',3'-*O*-isopropylidene-5'-*O*-trityl-D-ribosyl)-1,3-oxazinediones **13** and **14**, which after removal of the protecting groups gave oxazinomycin (**1**) and its α anomer **15**, respectively.

Much of the interest in C-nucleoside antibiotics[2,3] is due to their varied biological activities, which result from the close structural relationship of these substances to the "normal" nucleoside metabolites. Oxazinomycin (minimycin), **1**,[4,5] an illustrative example of this class of compounds, is elaborated by several *Streptomyces* species.[4-8] This antibiotic inhibits the growth of both gram-positive and gram-negative bacteria[6] and has shown significant activity against transplantable tumors.[4,6] Its structural similarity to uridine and pseudouridine is quite obvious. We now wish to describe a synthesis of oxazinomycin (1).



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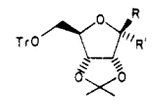


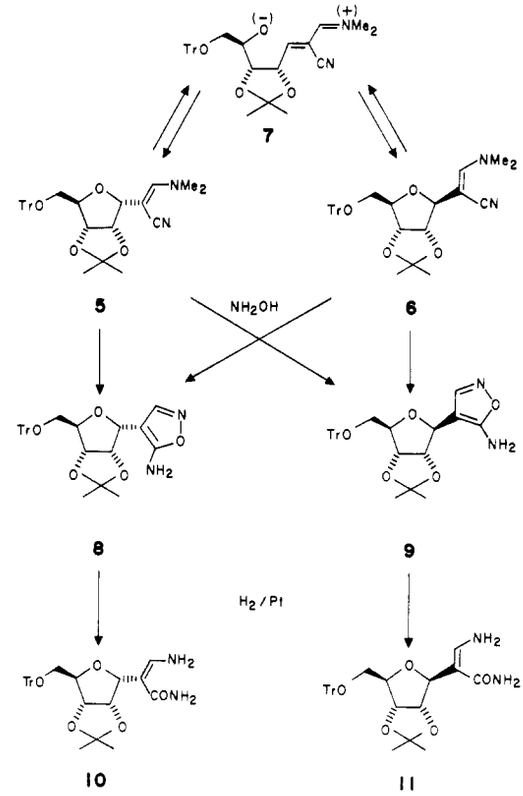
2 R,R' : OH, H
3 R : H; R' : CH_2CN
4 R : CH_2CN; R' : H
Tr = $C(Ph)_3$

A short time after this work had been initiated, the preparation of the epimeric ribosylacetonitriles **3** and **4** by Wittig reaction of 2,3-*O*-isopropylidene-5-*O*-trityl-D-ribose (**2**)[9] with cyanomethyltriphenylphosphorane was reported.[10] We prefer to obtain these compounds by the Horner modification, using sodium diethyl cyanomethylphosphonate in dimethoxyethane. Under these conditions, the reaction proceeds to completion at room temperature, affording a 1:2 mixture of **3** and **4**. Formylation with bis(dimethylamino)-*tert*-butyloxymethane,[11] whether performed on pure **3** or **4** or on the mixture of both, results in formation of the two 2-(1'-ribosyl)-3-dimethylaminoacrylonitriles **5** and **6** (2:1). Evidently, epimerization occurs readily between **3** and **4**,[9,10] as well as between **5** and **6**, presumably via opening and reclosure of the furanose ring, requiring base catalysis in the first case, and involving a dipolar intermediate **7** in the latter.

The assignment of the configuration at C-1' in **5** and **6** (as well as in subsequent intermediates) is based on ^1H NMR spectral data, in accord with literature precedence. Thus, Moffatt et al.[10] have convincingly demonstrated that in a series of *C*-glycosides derived from 2,3-isopropylidene-5-trityl-D-ribofuranose (**2**), including **3** and **4**, those with α configuration at the "anomeric" carbon consistently have values for the coupling constant $J_{3',4'}$ of 0–1 Hz, while in those with β configuration the magnitude of $J_{3',4'}$ is 4–5 Hz. This finding is the observable consequence of the preferred conformation of the heavily substituted tetrahydrofuran ring, which imposes a dihedral angle between H-3' and H-4' of ca. 90° in the α epimer and of ca. 160° in the β epimer.[10]

In agreement with this rule, the NMR signal (in $CDCl_3$), observed for H-4' of **5**, appears as a simple triplet at 4.22 ppm ($J_{3',4'}$ = 0 Hz), while H-4' of **6** gives rise to a quartet at 4.05 ppm ($J_{3',4'} = J_{4',5'}$ = 4 Hz). The ultraviolet spectra[12] of **5** and **6** are distinguished by an absorption maximum at ca. 275 nm (ϵ 17 000 and 18 000, respectively). It should be noted that the geometry around the acrylonitrile double bond, as drawn in **5** and **6**, is arbitrary, although according to published analogies,[13] the *Z* isomer would be expected to be much more preponderant.

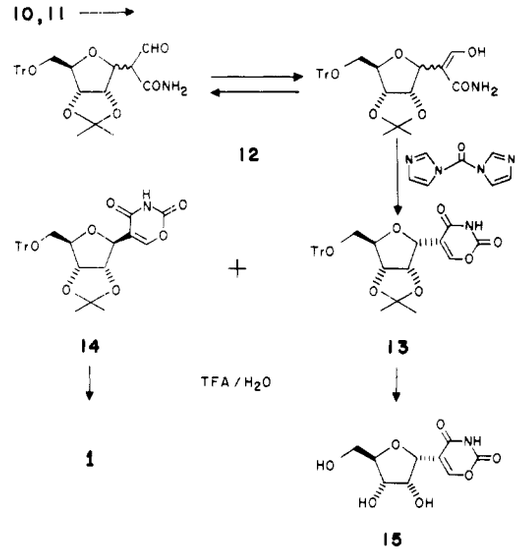
When the enamines **5** and **6** (individually or together) are allowed to react with hydroxylamine in DMF,[14] the aminoisoxazoles **8** and **9** are obtained. Both of these are characterized



by a uv absorption maximum at 247 nm (ϵ 8000). In agreement with the assigned stereochemistry, the NMR spectrum of **8** contains a triplet for H-4' (δ 4.06 ppm) and that of **9** has a doublet of triplets (quartet, δ 3.96 ppm, $J_{3',4'} = J_{4',5'}$ = 4 Hz). Catalytic hydrogenation of **8**, when carried out over Pt in dimethoxyethane, proceeds with consumption of 1 equiv of hydrogen to give the aminoacrylamide **10**. Analogously, reduction of **9** furnishes **11**. The ir spectra of both compounds (**10** and **11**) exhibit a strong amide band at 1660 cm^{-1}. Hydrolysis of the primary enamine function of **10**, or equally well

of **11**, is effected under mild conditions in a two-phase system consisting of 0.05 N aqueous hydrochloric acid and chloroform. From the NMR spectra, it is evident that the resulting 2-(1′-ribofuranosyl)-2-formylacetamide **12** is a mixture of aldehyde/enol tautomers, as well as of C-1′ epimers.

At this point, this seemingly circuitous sequence makes available the proper functionalities for the final steps of the oxazinomycin synthesis, without having required prohibitively harsh (basic) solvolysis conditions. Reaction of **12** with *N,N′*-carbonyldiimidazole (CDI) in dimethoxyethane, in the presence of a catalytic amount of base, completes the 1,3-oxazine heterocycle and furnishes the two epimeric products **13** and **14** (2:3). Upon aqueous workup of the reaction mixture,



some starting material is regenerated, presumably arising from the carbonylation product of that portion of enolized **12** which has *E* geometry. Separation of **13** and **14** is accomplished by preparative high-pressure liquid chromatography. The assignment of their respective stereochemistry is again possible with the help of the H-4′ NMR signal, which in **13** is observed as a triplet at δ 4.19 ppm, and in **14** as a quartet at δ 4.03 ppm ($J_{3',4'} = J_{4',5'} = 4$ Hz). The infrared spectra of both compounds contain a strong absorption band at 1790 cm^{-1}, which is characteristic for the oxazinedione system.[4] Removal of the protecting groups from **13** proceeds readily in 90% trifluoroacetic acid to afford the 1′-α epimer of oxazinomycin **15**. Analogous treatment of **14** gives oxazinomycin **1**. The physical properties of **15**, including ir, uv, and mass spectra, are very similar to those of **1**. Characteristic differences, however, exist in the NMR spectra of the two compounds, as expected. The properties of our synthetic **1** are in full agreement with those reported for oxazinomycin. In direct comparison by melting point, mixture melting point, and thin layer chromatography, our synthetic oxazinomycin is identical with material from a microbial source.[7]

Experimental Section

General. Melting points were taken on a Kofler hot stage melting point apparatus (Reichert) and are uncorrected. Infrared (ir) and ultraviolet (uv) spectra were recorded on Digilab FTS 14 and Cary Model 16 spectrophotometers, respectively. ^1H NMR spectra were obtained on Varian XL-100 and HA-100 instruments, and are reported in parts per million downfield from internal tetramethylsilane. Mass spectra were obtained ona CEC-110 mass spectrometer. Rotations were measured on a Perkin-Elmer 141 polarimeter. A Waters Associates Model ALC-202/R410 instrument was used for preparative high-pressure liquid chromatography.

Silica gel 60 (0.063–0.200 mm) and plates precoated with silica gel 60 F-254 (both from E. Merck) were used for column and thin layer chromatography, respectively.

Pyridine was distilled from BaO, and 1,2-dimethoxyethane (DME) from CaH_2. All the other solvents were dried with Davison 4A Molecular Sieves.

Some of the described experiments utilize extensive chromatography, most of which can be (and has been) omitted in routine runs, i.e. in the preparation of material to be used in the next step.

2′,3′-*O*-Isopropylidene-5′-*O*-trityl-D-ribofuranosylacetonitrile (3 and 4). To a suspension of 2.00 g (83.3 mmol) of NaH in 300 ml of dry DME (stirred under argon) was added dropwise (over 30 min) 17 ml (107.7 mmol) of diethyl cyanomethylphosphonate while cooling in an ice–water bath. After evolution of H_2 had ceased, the cooling was discontinued and 30 g (69.36 mmol) of 2,3-*O*-isopropylidene-5-*O*-trityl-D-ribose[9] in 200 ml of dry DME was added to the clear solution within 30 min. The reaction mixture was maintained for 2 h at room temperature under argon. It was then distributed between 2 l. of Et_2O and 1 l. of H_2O. The aqueous layer was extracted with 1 l. of Et_2O. The combined extracts were washed to neutrality with half-saturated brine (2 × 500 ml), diluted with benzene (500 ml), dried (Na_2SO_4), and evaporated to dryness in vacuo. The residual oil was dissolved in 40 ml of AcOEt–cyclohexane (1:4) and the solution chromatographed on a column (105 × 6 cm) containing 1.15 kg of silica gel. The column was developed with 5 l. of AcOEt–cyclohexane (1:4).

Early fractions afforded 9.92 g of crystalline **2,3-*O*-isopropylidene-5-*O*-trityl-α-D-ribofuranosylacetonitrile (3)**, mp 130 °C, from MeOH (reported[10] 130 °C); $[\alpha]^{25}D$ 10.1° (*c* 0.9982, $CHCl_3$) (reported[10] 9.8°). Further elution gave 14.32 g of **2,3-*O*-isopropylidene-5-*O*-trityl-β-D-ribofuranosylacetonitrile (4)**, contaminated with a minor amount of **3** (<10%), and finally 6.38 g of pure **4** as a colorless syrup, $[\alpha]^{25}D$ −5.5° (*c* 0.999, $CHCl_3$). The combined yield was 97%.

3-Dimethylamino-2-(2′,3′-*O*-isopropylidene-5′-*O*-trityl-D-ribosyl)acrylonitrile (5 and 6). A mixture (2:3) of **3** and **4** (30.5 g, 67 mmol) was dissolved in 250 ml of dry DMF and the solution was placed in a 500-ml flask fitted with a reflux condenser. An excess of bis(dimethylamino)-*tert*-butoxymethane[11] (45 ml) was added in one portion and the reaction mixture was stirred under argon for 3 h at 55 °C. The excess aminal ester and most of the solvent were evaporated in vacuo at 50 °C. The remaining dark syrup was taken up in $CHCl_3$ (ca. 30 ml) and the solution applied to a column containing 400 g of silica gel. The column was developed with $CHCl_3$ (200 ml) and $CHCl_3$–MeOH, 99.5:0.5 (2500 ml), the eluate being monitored by TLC ($CHCl_3$–MeOH, 98:2).

Fractions containing the epimeric mixtures of **5** and **6** were pooled. After evaporation of the solvents, the residue was dissolved in $CHCl_3$ (ca. 40 ml); dilution with Et_2O (300 ml) in portions yielded, after cooling, 17.38 g of crystalline **3-dimethylamino-2-(2′,3′-*O*-isopropylidene-5′-*O*-trityl-α-D-ribosyl)acrylonitrile (5)**. Mother liquors and washings (Et_2O) were evaporated in vacuo. The light brown residue, dried at 50 °C (0.01 mmHg), was purified by chromatography on 540 g of silica gel. The column, packed in $CHCl_3$, was eluted with $CHCl_3$–MeOH, 99:1 (1500 ml) and 98:2 (3000 ml). The oil obtained from the early fractions was taken up in Et_2O. Upon concentration of the solution to ca. 25 ml, dilution with cyclohexane (60 ml) in portions, and cooling, 6.36 g of pure **3-dimethylamino-2-(2′,3′-*O*-isopropylidene-5′-*O*-trityl-β-D-ribosyl)acrylonitrile (6)** was obtained (as a solvate with 1 mol of cyclohexane).

Later fractions contained an epimeric mixture of **5** and **6**. Fractional crystallization of the residue gave another 1.43 g of the α epimer **5** (total 18.82 g) from $CHCl_3$–Et_2O, then an additional 1.60 g of solvated β epimer **6** from Et_2O–cyclohexane. A further amount of crystalline **6** (1.66 g, 9.62 g in total) could be isolated by chromatography of the mother liquors on 400 g of silica gel in AcOEt–cyclohexane, 3:7. The epimer **5** had mp 180–181.5 °C; $[\alpha]^{25}D$ −51.8° (*c* 0.9856, $CHCl_3$); uv (EtOH) infl 230 nm (ε 11 500), max 276 (16 900); ir ($CHCl_3$) 2810, 2180, 1634, 1107, 1074, 708 cm^{-1}; NMR ($CDCl_3$) δ 1.33 and 1.56 [2 s, $C(CH_3)_2$], 3.11 [s, $N(CH_3)_2$], 3.20 (ddd, CH_2OTr), 4.22 (t, H-4′), 4.56 (t, H-2′), 4.64 (2 d H-1′ and H-3′), 6.61 (s, vinylic), 7.20–7.55 (m, 15, aromatic).

Anal. Calcd for $C_{32}H_{34}N_2O_4$: C, 75.27; H, 6.71; N, 5.49. Found: C, 75.11; H, 6.46; N, 5.50.

The epimer **6** had mp 78–83 °C; $[\alpha]^{25}D$ −28.7° (*c* 0.9917, $CHCl_3$); uv (EtOH) infl 230 nm (ε 11 500), max 274–275 (18 200); ir ($CHCl_3$) 2815, 2190, 1637, 1075, 708 cm^{-1}; NMR ($CDCl_3$) δ 1.32 and 1.53 [2 s, $C(CH_3)_2$], 3.06 [s, $N(CH_3)_2$], 3.28 (d, OCH_2Tr), 4.05 (q, H-4′, J = 4 Hz), 4.15 (d, H-1′, J = 5 Hz), 4.51 (dd, H-3′), 4.66 (dd, H-2′), 6.55 (s, vinylic), 7.20–7.60 (m, 15, aromatic).

Anal. Calcd for $C_{32}H_{34}N_2O_4 \cdot C_6H_{12}$: C, 76.74; H, 7.80; N, 4.71. Found: C, 76.81; H, 8.14; N, 4.79.

The combined yield was 79.2%.

5-Amino-4-(2′,3′-*O*-isopropylidene-5′-*O*-trityl-D-ribosyl)-isoxazole (8 and 9). A mixture (2:1) of **5** and **6** (30.69 g, 57.34 mmol) was dissolved in 300 ml of dry DMF. To this solution was added 50 ml of dry pyridine and 5.10 g (73.3 mmol) of NH_2OH·HCl. Upon stirring at 68–70 °C under argon, a clear solution was obtained within 15 min. The reaction was kept at 70 °C for 6.5 h while monitoring by TLC (Et_2O–cyclohexane, 10:3). The solvents were then evaporated in vacuo at 45 °C and the residual syrup was distributed between $CHCl_3$ (1200 ml) and H_2O (600 ml). The aqueous layer was extracted with a second portion of $CHCl_3$ (600 ml). The organic extracts were combined, washed with half-saturated brine (3 × 400 ml), dried (Na_2SO_4), and evaporated in vacuo. The residual syrup was chromatographed on 400 g of silica gel, successively with $CHCl_3$–MeOH, 98.5:1.5 (900 ml) and 98:2 (1500 ml). Early fractions were rechromatographed on 600 g of silica gel ($CHCl_3$–MeOH, 98:2, 3500 ml) to give 0.610 g of unreacted **5** and **6**, 2.726 g of crystalline **5-amino-4-(2′,3′-*O*-isopropylidene-5′-*O*-trityl-β-D-ribosyl)isoxazole (9)** (from Et_2O–petroleum ether, 30–60 °C) as a solvate with 1 mol of Et_2O, and 4.432 g of **5-amino-4-(2′,3′-*O*-isopropylidene-5′-*O*-trityl-α-D-ribosyl)isoxazole (8)** as an amorphous foam.

Later fractions, upon rechromatography on 1.1 kg of silica gel (Et_2O–cyclohexane, 10:3, 4500 ml), yielded an additional 0.985 g of crystalline **9** and 11.211 g of **8**.

The still unresolved fractions were further chromatographed on silica gel (Et_2O–cyclohexane, 10:3) to give in total 19.608 g of **8** as a white foam and 5.638 g of **9** (solvated with 1 mol of Et_2O). The combined yield was 85.8%.

Pure **8** had $[\alpha]^{25}D$ −11.5° (*c* 0.9960, $CHCl_3$); uv (EtOH) max 247 nm (ε 8000); ir ($CHCl_3$) 3490, 3390, 1646, 1505, 1495, 1105, 1074, 706 cm^{-1}; NMR (Me_2SO-d_6) δ 1.24, 1.44 [2 s, $C(CH_3)_2$], 3.10 (m, CH_2OTr), 4.06 (t, H-4′), 4.54 (q, H-2′), 4.62 (d, H-3′), 4.72 (d, H-1′), 6.54 (broad s, exchangeable, NH_2), 7.20–7.50 (m, 15 aromatic), 8.01 (s, =CH–).

Anal. Calcd for $C_{30}H_{30}N_2O_5$: C, 72.27; H, 6.07; N, 5.61. Found: C, 71.49; H, 6.07; N, 5.67.

Pure **9**, a solvate with 1 mol of Et_2O, had mp 90–96 °C; $[\alpha]^{25}D$ −13.4° (*c* 0.9890, $CHCl_3$); uv (EtOH) max 248 nm (ε 8400); ir 3485, 3370, 1653, 1514, 1505 (w), 1080, 707 cm^{-1}; NMR (Me_2SO-d_6) δ 1.09 (t, CH_3 of Et_2O), 1.27, 1.49 [2 s, $C(CH_3)_2$], 3.13 (d, CH_2OTr), 3.37 (q, CH_2 of Et_2O), 3.96 (q, H-4′), 4.50–4.78 (m, H-3′, H-2′, H-1′), 6.78 (broad s, $-NH_2$), 7.16–7.52 (m, 15, aromatic), 8.10 (s, =CH–).

Anal. Calcd for $C_{30}H_{30}N_2O_5$·$C_4H_{10}O$: C, 71.31; H, 7.04; N, 4.89. Found: C, 71.29; H, 7.15; N, 4.84.

3-Amino-2-(2′,3′-*O*-isopropylidene-5′-*O*-trityl-α-D-ribosyl)acrylamide (10). A solution of 5.01 g (10.05 mmol) of **8** in 75 ml of dry DME was hydrogenated at room temperature in the presence of 250 mg of PtO_2. The consumption of H_2 was 285 ml within 25 min (theoretical 274 ml). The mixture was stirred under nitrogen with a small amount of decolorizing carbon and filtered through a pad of Celite. After evaporation of the solvents in vacuo at <30 °C, the residue was taken up in 250 ml of $CHCl_3$. The solution was washed with 2 × 200 ml of H_2O, dried (Na_2SO_4), and evaporated under reduced pressure. Upon drying at 0.005 mmHg at room temperature for 2 days and at 50 °C for 2 h, 5.20 g (90%) of **10** was obtained as a white foam: $[\alpha]^{25}D$ −27.2 (*c* 0.8655, $CHCl_3$); uv (EtOH) max 271 nm (ε 10 000); ir ($CHCl_3$) 3510, 3375, 1660, 1576, 1100, 1070, 705 cm^{-1}; NMR (Me_2SO-d_6) δ 1.18, 1.34 [2 s, $C(CH_3)_2$], 2.80–3.25 (m, CH_2OTr), 4.03 (t, H-4′), 4.32–4.64 (m, H-1′, H-2′, H-3′), 6.20 (broad s, NH_2), 6.72 (t, =CH–; s, upon D_2O exchange), 7.00–7.64 (m, 15, aromatic and NH_2, exchange), 8.24 ($CHCl_3$).

Anal. Calcd for $C_{30}H_{32}N_2O_5$·0.65$CHCl_3$: C, 63.67; H, 5.69; N, 4.85. Found: C, 63.83; H, 5.80; N, 4.53.

3-Amino-2-(2′,3′-*O*-isopropylidene-5′-*O*-trityl-β-D-ribosyl)acrylamide (11), prepared analogously by hydrogenation of **9**, was a white foam from $CHCl_3$: $[\alpha]^{25}D$ −12.7° (*c* 0.8694, $CHCl_3$); uv (EtOH) max 273 nm (ε 12 100); ir ($CHCl_3$) 3510, 3485, 3355, 1665, 1580, 1100, 706 cm^{-1}; NMR (Me_2SO-d_6) δ 1.26, 1.46 [2 s, $C(CH_3)_2$], 3.21 (m, CH_2OTr), 3.87 (q, H-4′), 4.14 (q, H-2′ or H-3′), 4.60 (m, H-1′ and H-2′ or H-3′), 6.26 (broad s, NH_2, exchange), 6.81 (t, =CH–, collapses slowly to a s upon D_2O exchange), 7.10–7.55 (m, 15, aromatic and NH_2), 8.29 ($CHCl_3$).

Anal. Calcd for $C_{30}H_{32}N_2O_5$·0.65$CHCl_3$: C, 63.67; H, 5.69; N, 4.85. Found: C, 63.99; H, 5.93; N, 4.67.

2-Formyl-2-(2′,3′-*O*-isopropylidene-5′-*O*-trityl-D-ribosyl)-acetamide (12). A solution of **10** (4.218 g) in 250 ml of $CHCl_3$ was vigorously stirred for 7 h at room temperature together with 500 ml of 0.05 N HCl. Then 250 ml of $CHCl_3$ was added and the layers were separated. The aqueous phase was extracted with 250 ml of $CHCl_3$. The combined extracts were washed with 3 × 300 ml of H_2O, dried (Na_2SO_4), and evaporated in vacuo at <35 °C. The residue was dissolved in 10 ml of AcOEt–Et_2O, 1:1, and the solution was chromatographed on 400 g of silica gel with AcOEt–Et_2O, 35:65 (2500 ml). The residue obtained from the first fractions (0.540 g) was enriched in the α epimers (α/β ca. 2:1, as determined by integration of the CHO protons in NMR), while the subsequent eluate gave a white foam (2.415 g) in which the β epimers were largely predominant (α/β ca. 2:9). This material (2.955 g, 70%) was used in the next step without any further purification.

Both fractions had very similar spectral properties: uv (EtOH) max 266 nm (ε 2600); (0.1 N KOH) sh 230 nm (9600), max 270 (12 300); ir ($CHCl_3$) 3480, 3345, 1710 (w), 1655 cm^{-1}; (pyridine) 1728, 1690, 1658 cm^{-1}; NMR (partial, Me_2SO-d_6) δ 4.07 (q, H-4′ of β epimers), 4.33 (t, H-4′ of α epimers), 9.58–9.72 (m, CHO, integration for less than one proton, indicating presence of tautomeric forms).

5-(2′,3′-*O*-Isopropylidene-5′-*O*-trityl-D-ribosyl)-1,3-oxazine-2,4-dione (13 and 14). To a stirred suspension of 353 mg of KH (22.5% in oil, 2 mmol) in 20 ml of dry DME was added dropwise at 10 °C a solution of 2.48 g (4.94 mmol) of **12** in 25 ml of DME. After evolution of H_2 had ceased, 1.62 g (10 mmol) of 1,1′-carbonyldiimidazole dissolved in 35 ml of DME was added dropwise at 10 °C to the clear solution. The reaction mixture was stirred under argon at room temperature for 6 h. It was then diluted with 500 ml of Et_2O and 175 ml of cold 0.15 N HCl. The aqueous layer was extracted with a second portion of Et_2O. The organic extracts were washed with H_2O (4 × 100 ml), diluted with 150 ml of benzene, dried (Na_2SO_4), and evaporated at 35 °C under reduced pressure. The residue was dissolved in 8 ml of AcOEt–Et_2O, 35:65, and the solution chromatographed on 400 g of silica gel with 2000 ml of the same solvent mixture. Epimeric 5-(2′,3′-*O*-isopropylidene-5′-*O*-trityl-D-ribosyl)-1,3-oxazine-2,4-dione (**13** and **14**, 1.245 g, 47.7%) was eluted first. Starting material (0.535 g) was recovered from later fractions, giving an actual yield of 64%.

Partial separation of the epimers was achieved by column chromatography. Thus, 3.20 g of a mixture of **13** and **14** was dissolved in 10 ml of AcOEt–Et_2O–cyclohexane, 30:10:60, and the solution applied to a column (80 × 4.6 cm) packed with 600 g of silica gel. Elution with AcOEt–Et_2O–cyclohexane, 30:10:60 (4000 ml, 1.5 ml/min), afforded 0.897 g of the less polar **5-(2′,3′-*O*-isopropylidene-5′-*O*-trityl-α-D-ribosyl)-1,3-oxazine-2,4-dione (13)** as an amorphous, white powder, which after evaporation from AcOEt–*n*-heptane and drying at 70 °C (0.005 mmHg) for 24 h had $[\alpha]^{25}D$ −48.4° (*c* 1.0015, $CHCl_3$); uv (EtOH) infl 230 nm (ε 12 700), 259 (980), 270 (480); ir ($CHCl_3$) 3390, 1790, 1757, 1725, 1705, 1080, 708 cm^{-1}; NMR (Me_2SO-d_6) δ 1.23, 1.33 [2 s, $C(CH_3)_2$], 3.16 (d, CH_2OTr), 4.19 (t, H-4′), 4.66 (d, H-3′), 4.87 (t, H-2′), 4.93 (d, H-1′), 7.20–7.50 (m, 15, aromatic), 7.64 (s, =CH–), 12.02 (s, exchanges, NH).

Anal. Calcd for $C_{31}H_{29}NO_7$: C, 70.58; H, 5.54; N, 2.65. Found: C, 70.59; H, 5.49; N, 2.71.

The residue obtained from the remaining fractions could be resolved into its components by preparative high-pressure liquid chromatography. Thus, batches of ca. 500 mg of the mixture were chromatographed on an 8 ft × 0.375 in. column packed with Porasil A, using AcOEt–*n*-heptane, 1:4, as the eluent. Two recycles provided complete separation of the epimers. After evaporation of the fractions in vacuo, the residues were dried at 70 °C (0.005 mmHg) for 24 h to give an additional 0.350 g of **13** and 1.771 g of **5-(2′,3′-*O*-isopropylidene-5′-*O*-trityl-β-D-ribosyl)-1,3-oxazine-2,4-dione (14)** as a white, amorphous powder: $[\alpha]^{25}D$ 8.4° (*c* 0.9912, $CHCl_3$); uv (EtOH) infl 230 nm (ε 13 400), 260 (950), 270 (450); ir ($CHCl_3$) 3390, 1790, 1760, 1725, 1085, 710 cm^{-1}; NMR (Me_2SO-d_6) δ 1.25, 1.47 [2 s, $C(CH_3)_2$], 3.13 (d, CH_2OTr), 4.03 (q, H-4′), 4.55 (t, H-3′), 4.64–4.83 (m, H-1′, H-2′), 7.20–7.50 (m, 15 aromatic), 7.79 (s, =CH–), 12.01 (s, exchanges, NH).

Anal. Calcd for $C_{31}H_{29}NO_7$: C, 70.58; H, 5.54; N, 2.66. Found: C, 70.63; H, 5.72; N, 2.66.

5-α-D-Ribofuranosyl-1,3-oxazine-2,4-dione (15). A solution of 791 mg (1.50 mmol) of **13** in 25 ml of 90% CF_3COOH was stirred at room temperature for 2.5 h. The solvents were removed at ca. 30 °C (0.2 mmHg) and the residue was dried azeotropically by evaporation from absolute EtOH. The resulting solution was triturated with 25 ml of benzene and the suspension stirred at room temperature for 1 h. The insoluble matter was collected by filtration and washed with several small portions of Et_2O to give 368 mg (100%) of **15** as a white, crystalline powder, mp 163–167 °C dec (with previous softening), pure by TLC.

Recrystallization from a small volume of MeOH–H_2O (5:1) afforded 250 mg of needles: mp 168–170 °C; $[\alpha]^{25}D$ −82.6 (*c* 0.9918, H_2O); uv max (H_2O) 230 nm (ε 4420); ir (KBr) 3400, 3320, 1795, 1770, 1690, 1675, 1653 cm^{-1}; NMR (D_2O) δ 3.74, 3.97 (CH_2, 2 dd, J_{vic} = 2.5, 5, J_{gem} = 12.5 Hz), 4.04 (H-4′, ddd, *J* = 2.5, 5, 7.5 Hz), 4.33 (H-3′, dd, *J* = 4, 7.5 Hz), 4.46 (H-2′, dd, *J* = 4, 3 Hz), 5.12 (H-1′, dd, *J* = 3, 1.5 Hz), 7.77 (vinylic, d, *J* = 1.5 Hz); MS *m/e* 245 (M^+), 227 (M − H_2O), 201 (M −

CO_2), 184 (M − HNCO), 140, 112.

Anal. Calcd for $C_9H_{11}NO_7$: C, 44.09; H, 4.52; N, 5.71. Found: C, 43.98; H, 4.40; N, 5.58.

5-β-D-Ribofuranosyl-1,3-oxazine-2,4-dione (Oxazinomycin, 1). A solution of 1.298 g (2.46 mmol) of **14** in 35 ml of 90% CF_3COOH was stirred at room temperature for 3 h. The solvents were removed at ca. 30 °C (0.2 mmHg). The residue was dried azeotropically by evaporation from absolute EtOH and purified by chromatography on 200 g of silica gel. The column was developed with AcOEt–AcMe–MeOH–H_2O, 70:10:5:5, and appropriate fractions were evaporated in vacuo at 30 °C. Crystallization of the residue from MeOH containing a small amount of H_2O afforded 387 mg of oxazinomycin (**1**), mp 153–155 °C. The mother liquors, after evaporation, gave an additional 98 mg of **1** from AcMe, mp 152–154 °C, total yield 80%, mmp with an authentic sample[7] 153–155 °C. Occasionally, upon slow recrystallization from water–methanol, a second polymorph was obtained, which had mp 161–162 °C dec (reported[4–6] 161 °C). Synthetic **1** and natural oxazinomycin had identical R_f values in several TLC systems; e.g., in EtOAc–AcMe–H_2O, 70:10:5:5, the R_f was 0.34: $[\alpha]^{25}D$ +15.29° (*c* 0.9942, H_2O); uv max (H_2O) 230 nm (ε 4700); ir (KBr) 3470, 3420, 1797, 1773, 1678 cm^{-1}; NMR (D_2O) δ 3.77, 3.90 (CH_2, 2 dd, J_{vic} = 5, 3, J_{gem} = 12.5 Hz), 4.06 (H-4′, ddd, *J* = 5,3,5 Hz), 4.19 (H-3′, t, *J* = 5, 5 Hz), 4.34 (H-2′, t, *J* = 5, 5 Hz), 4.72 (H-1′, d, *J* = 5 Hz), 7.88 (vinylic, s); MS *m/e* 227 (M − H_2O), 209, 202, 196.

Anal. Calcd for $C_9H_{11}NO_7$: C, 44.09; H, 4.52; N, 5.71. Found: C, 44.22; H, 4.52; N, 5.70.

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Carbon-13 Nuclear Magnetic Resonance Studies of Fungal Metabolites, Aflatoxins, and Sterigmatocystins

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^{13}C NMR spectra are reported for 12 of the fungal metabolites which contain the fused bisdihydrofuran ring system and are produced by certain strains of *A. flavus*, *A. parasiticus*, and *A. versicolor*. Included are the aflatoxins B_1, B_2, B_{2a}, B_3 (parasiticol), D_1, G_1, G_2, and G_{2a} and sterigmatocystin, dihydrosterigmatocystin, *o*-methylsterigmatocystin, and *o*-methyldihydrosterigmatocystin. Chemical shifts have been assigned on the basis of known substituent effects, off-resonance decoupling experiments, and comparison among the related compounds.

The aflatoxins and related sterigmatocystins, fungal metabolites produced by certain strains of *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus versicolor*, are of considerable interest because of their widespread occurrence in human and animal foodstuffs and their carcinogenic effects in all laboratory animals with which they have been tested.[1,2] The common structural feature of these compounds is the bisfuran ring system, which in the aflatoxins is fused to a substituted coumarin structure. Previous studies have shown that the above compounds are derived biosynthetically from a polyketide (acetate) precursor[3–7] and that sterigmatocystin is a precursor of aflatoxin B_1.[8] Although the structures of these compounds have been elucidated previously,[1] the recent advances in ^{13}C NMR toward smaller sample sizes and the wealth of information available from ^{13}C NMR[9] makes ^{13}C NMR a valuable tool for the identification of these metabolites and the structure determination of future metabolites.

Two reports[4,5] have appeared recently on the ^{13}C NMR spectrum of aflatoxin B_1 in connection with ^{13}C labeling studies into the biosynthetic origin of aflatoxin B_1. However, the two reports differ in their assignment of the carbon chemical shifts of aflatoxin B_1. In view of the importance of the aflatoxins and related compounds, we wish to report here our studies of the ^{13}C NMR spectra of eight related aflatoxins. A consistent assignment of the ^{13}C chemical shifts of the aflatoxins is made which is in agreement with one of the previous assignments.[4] In addition, ^{13}C NMR data are also reported for sterigmatocystin and three derivatives. The results are consistent with a previous assignment of the ^{13}C NMR spectrum of sterigmatocystin.[7]

Experimental Section

Natural abundance, proton-decoupled ^{13}C NMR spectra were obtained on a JEOL PFT-100 spectrometer equipped with the JEOL EC-100 data system. Fourier transform spectra were obtained using spectral widths of 5000 and 6250 Hz, with 8K data points. A pulse angle of ~40° was used with a repetition rate of 3 s. Chemical shifts are reported in parts per million downfield from internal tetramethylsilane and are considered accurate to 0.1 ppm. Single frequency, off-resonance proton decoupled (sford) spectra were obtained on each sample.