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A TOTAL SYNTHESIS OF DISTAMYCIN A, AN ANTIVIRAL ANTIBIOTIC

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Abstract—A new total synthesis of distamycin A (1) is described. The route followed passes through an intermediate acid (2a) which can serve as a convenient starting material for the synthesis of distamycin analogs.

There are few antiviral drugs in use today.[1] It seems, therefore, of some importance to follow the known leads in this field with the aim of establishing structure-activity relationships which may help develop new drugs. Distamycin A (1) is a known[2] antiviral antibiotic with strong activity (mostly measured *in vitro*) against DNA and oncorna viruses.[3] It has been used topically in cases of herpes infections. Although a considerable amount of biological and biochemical work on this and related antibiotics has been reported,[3] only limited information has been published on the structural requirements for antiviral activity.[4] This is in particular true for modifications in the alkyl amidine side chain. The reason for the lack of such synthetic work is due, in part, to the fact that the only published synthesis of distamycin[5] does not allow for the facile preparation of analogs and each alkyl amidine side chain homolog has to be prepared by a separate total synthesis. In order to overcome this difficulty we have developed a new synthesis in which a single intermediate (the acid 2a) can serve as starting material for numerous side chain homologs. In the present paper we present the details of this synthesis. Later we shall report on the synthesis and antiviral evaluation of distamycin analogs.

The synthetic route followed by us is outlined in the Scheme. The starting material used was the commercially available N-methyl-2-carboxy-pyrrole (3) which was nitrated and esterified. Separation by column chromatography gave the known methyl esters of N-methyl-4-nitro-pyrrole carboxylic acid (4)[6,7] and its 5-nitro isomer. The ester (4) was reduced to the amine (5) which without isolation or purification was condensed with N-methyl-4-nitropyrrole-2-carboxyl chloride (6)[7] to give the amide-ester (7). The reduction-condensation process was then repeated, leading to the intermediate nitro-ester (2b), which on mild hydrolysis led to the important acid (2a). The overall yield from the monocyclic nitro-ester (4) to the three-cyclic nitro acid (2a) was 33%.

Compounds 2a and 2b melt above 270° and are insoluble in most organic solvents except dimethyl formamide (DMF) or dimethylsulfoxide (DMSO). Their mass spectra show a prominent, typical peak at *m/e* 275 which is probably due to the ion a.

Condensation of the acyl chloride of 2a with β-amino propionitrile (8) was not successful; however direct reaction of the acid (2a) with 8 in the presence of both dicyclohexyl carbodiimide (DCC) and benzyhydroxy-triazole gave the desired nitrile (9)[5] which like 2a and 2b exhibits the typical mass ion peak at *m/e* 275. The nitrile (9) was converted into the known nitroamidino compound (10)[5] by the standard reaction with hydrochloric acid followed by ammonia. On reduction the amino-amidino compound (11) was obtained, which due to its sensitivity and instability was directly condensed[8] with formic acid in the presence of DCC to give distamycin A (1). Synthetic distamycin was directly compared with natural distamycin by IR, UV, NMR and mass spectrometry. The two samples had identical spectra.

Except for the last step, in which the yield was 15% only, all steps from the acid 2a to distamycin A were good and emphasize the overall practical nature of the above total synthesis.

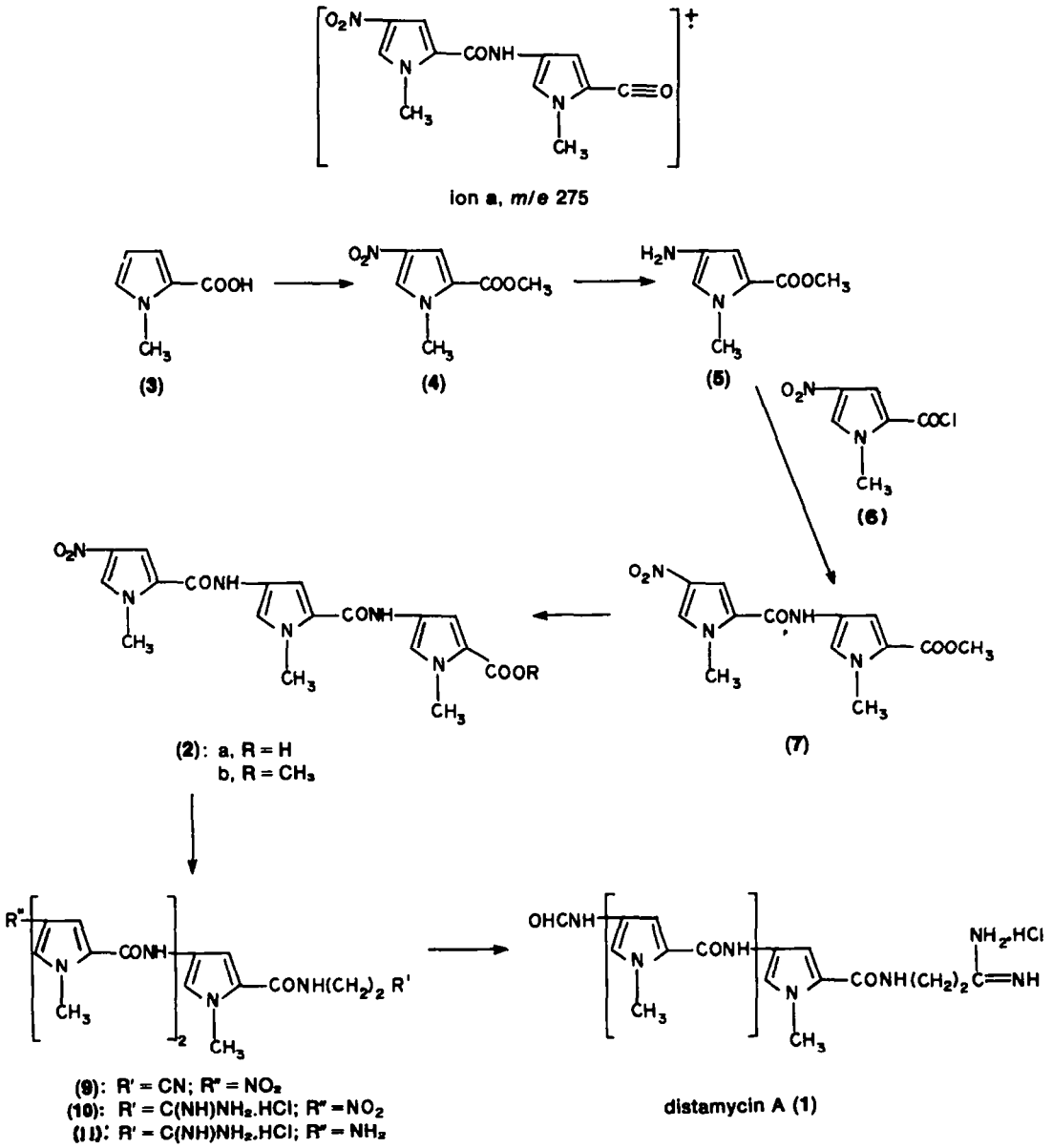
EXPERIMENTAL

Unless otherwise stated, the following apply. UV measurements were made for solns in EtOH. IR spectra were taken for solns in $CHCl_3$; for insoluble compounds—in KBr pellets. ^1H NMR data were determined in $CDCl_3$ or CCl_4 with Me_4Si as internal standard. In some cases DMSO(d_6) was used as solvent with 3'(trimethylsilyl)-tetradeutero sodium propionate as external standard. Tlc was performed on 0.3 mm silica gel plates, which were developed with Ehrlich reagent (2% N-dimethyl-aminobenzaldehyde in 6 N HCl) or by irradiation at 254 nm. Column chromatography was performed on silica gel (Merck, Kieselgel 60, 70–230 mesh). Mass spectra were obtained by direct inlet at 70 eV; in several cases the technique of field desorption (F.D.) was used.

N-Methyl-4-nitropyrrole-2-carboxylic acid methyl ester (4). Compound 3 (5 g) was dissolved in Ac_2O (30 ml). The soln was cooled to −25°. Cold HNO_3 (70%; 4 ml) in Ac_2O (19 ml) was slowly added. The soln was stirred (still at −25°) for 30 min, then poured into ice and the mixture was stirred for 30 min. The mixture was extracted with $CHCl_3$, dried and evaporated. A cold soln of H_2SO_4 (5 ml) in MeOH (50 ml) was added to the residue. The mixture was boiled under reflux for 12 hr. Water was added and the mixture was extracted with $CHCl_3$. The organic layer was dried and evaporated. The residue was chromatographed on alumina (activity II). Elution with petroleum ether:ether (95:5) gave N-methyl-5-nitropyrrole-2-carboxylic acid methyl ester (800 mg, 10.6% yield), m.p. 114° (lit.[6] m.p. 112°); δ($CDCl_3$) 3.84 (–$COOCH_3$), 4.27 (N–CH_3), 6.84 (1 H, d, J = 4.5 Hz), 7.07 (1 H, d, J = 4.5 Hz).

Further elution with petroleum ether: ether (8:2) gave 4 (2.8 g, 42%), m.p. 122° (lit.[6] 120°), δ($CDCl_3$) 3.84(–$COOCH_3$), 4.0 (N–CH_3), 7.34 (1 H, d, J = 1.5 Hz), 7.55 (1 H, d, J = 1.5 Hz); λ_{max}^{EtOH} 237, 296 nm (ε, 18400, 6900); ν($CHCl_3$) 1710 cm^{-1}: *m/e* 184 (M^+ 100%), 153 (76%), 107 (30%) (Found: C, 45.8; H, 4.13; N, 14.7. $C_7H_8N_2O_4$ requires: C, 45.6; H, 4.9; N, 15.2%).

N - Methyl - 4 - (N - methyl - 4 - nitropyrrole - 2 - carboxy-amido) - pyrrole - 2 - carboxylic acid methyl ester



(7). Compound 4 (1 g) was dissolved in dimethyl formamide (12 ml) and was reduced over Pd on charcoal (300 mg) at atmospheric pressure. Water (45 ml) was added and the catalyst was filtered off. $NaHCO_3$ (550 mg) was introduced into the filtrate, followed by a soln of 6 prepared[7] from N-methyl-4-nitropyrrole-2-carboxylic acid (1 g) with thionyl chloride (3 ml) in benzene (5 ml). The mixture was stirred overnight and filtered. The crystalline product obtained was washed with $NaHCO_3$, water and MeOH. Compound 7 (1.3 g; 75%) had m.p. 262°; δ(DMSO-d_6) 3.7 ($COOCH_3$), 3.8, 3.9 (2 N-CH_3), 6.83, 7.4, 7.5, 8.12 (4 arom. H'S, each with J = 1.5 Hz), 9.24 (amide H); λ_{max}^{DMF} 288 nm (ϵ, 24500); ν(KBr) 1690, 1660 cm^{-1}; *m/e* 306 (M^+, 50%), 275 (5%), 153 (100%), 136 (5%) (Found: C, 50.78; H, 4.77; N, 17.91. $C_{13}H_{15}N_4O_4$ requires: C, 50.98; H, 4.88; N, 18.24%).

N - *Methyl* - 4 - [N - *methyl* - 4 - (N - *methyl* - 4 - *nitropyrrole* - 2 - *carboxyamido*) - *pyrrole* - 2 - *carboxamido*] - *pyrrole* - 2 - *carboxylic acid methyl ester* (2b). The methyl ester 7 (1 g) was dissolved in dimethyl formamide (20 ml) and was reduced with Pd on charcoal (300 mg) at atmospheric pressure. Water (80 ml) was added and the catalyst was filtered off. $NaHCO_3$ (300 mg) was introduced into the filtrate, followed by a soln of 6 (0.6 g) in benzene (5 ml). The mixture was stirred overnight. The ppt was filtered off and washed with $NaHCO_3$aq, water and MeOH. Compound 2b (730 mg; 52%) thus obtained had a m.p. > 270°; δ(DMSO-d_6), 3.72 ($COOCH_3$), 3.85, 3.86, 3.96 (3N-CH_3), 6.89, 7.05, 7.26, 7.46, 7.57, 8.12 (6H, each one d, J = 1.5), 9.95, 10.27 (2H, s, amide); λ_{max}^{DMF} 298 nm (ϵ, 38400); ν(KBr) 1690, 1650 cm^{-1}; *m/e* 428 (48%), 397 (24%), 275 (100%) (Found: C, 52.11; H, 4.73; N, 18.7. $C_{19}H_{20}N_6O_6$ requires: C, 53.27; H, 4.67; N, 19.62%).

Hydrolysis of the methyl ester 2b *to the acid* 2a. The above described 2b (1 g) was suspended in EtOH (40 ml). A soln of NaOH (800 mg) in water (40 ml) was added and the mixture was boiled under reflux until a clear soln was obtained. The soln was cooled, filtered and the filtrate was acidified with 6 N HCl. A yellow ppt was formed, which was filtered off and washed with water and MeOH. Compound 2a (830 mg; 86%) thus obtained had a m.p. > 270°; δ(DMSO-d_6) 3.82, 3.92 (3 N-CH_3), 6.82, 7.01, 7.19, 7.30, 7.51, 8.97 (6 arom. H), 9.48, 9.65 (2H, amide); λ_{max}^{DMF} 297 nm (ϵ, 35700); ν(KBr) 1700, 1660 cm^{-1}; *m/e* 414 (M^+, 12%), 370 (75%), 340 (20%), 275 (100%) (Found: C, 51.98; H, 4.58; N, 20.78. $C_{18}H_{18}N_6O_6$ requires: C, 52.17; H, 4.34; N, 20.29%).

N - *Methyl* - 4 - [N - *methyl* - 4 - (N - *methyl* - 4 - *nitropyrrole* - 2 - *carboxyamido*) - *pyrrole* - 2 - *carboxyamido*] - *pyrrole* - 2 - *carboxyamido* - β - *propionitrile* (9). Acid 2a (700 mg) was

dissolved in dimethylformamide (15 ml). β-Aminopropionitrile (178 mg) was added, followed by addition of benzhydroxytriazole (460 mg) and dicyclo-hexylcarbodiimide (350 mg) in dimethylformamide (2 ml). The soln was stirred for 1 h at 0° and then overnight at room temperature, filtered and water was slowly added. The yellow ppt formed was filtered off and crystalized from MeOH to give **9** (350 mg; 68%), m.p. 257–260° (lit.[5] 282–5°); σ(DMSO-d_6) 2.78 (2 H, tr), 3.70 (2 H, tr), 3.87, 3.90, 4.0 (3 N–CH_3), 7.0, 7.11, 7.25, 7.26, 7.62, 8.11 (6 H, arom), 8.38, 9.67, 10.4 (3 H, amide); λ_{max}^{DMF} 295 nm (ε, 27500); ν(KBr) 2220, 1640 cm^{-1}; *m/e* 466 (M^+, 39%), 413 (2%), 344 (10%) 275 (100%) (Found: C, 53.18; H, 5.18; N, 23.08; $C_{21}H_{22}N_8O_5$. $1/2H_2O$ requires: C, 53.18; H, 5.18; N, 23.08%).

N - *Methyl* - 4 - [N - *methyl* - 4 - (N - *methyl* - 4 - *nitropyrrole* - 2 - *carboxyamido*) - *pyrrole* - 2 - *carboxyamido*] - *pyrrole* - 2 - *carboxyamido* - β - *propionamidin, hydrochloride* (**10**). This compound was prepared as described by Penco *et al.*[5] The following physical data were recorded, m.p. 204–7°; δ(DMSO-d_6) 2.7 (2 H, tr), 3.60, 3.80, 3.98 (3 N–CH_3), 6.94, 7.10, 7.36, 7.59, 7.78 (6 H, arom), 8.83 (mult, amidine H's), 9.81, 10.02, 10.19 (H, amides); λ_{max}^{EtOH} 240, 295 nm (ε 29300, 35000); ν(KBr) 1640 cm^{-1}; *m/e* 466 (M^+–NH_3, 38%), 413 (15%), 370 (10%), 344 (30%), 291 (50%), 275 (100%), 248 (42%).

Distamycin A (1). The amidine hydrochloride **10** (0.9 g) was dissolved in dimethylformamide and was reduced at atmospheric pressure with Pd on charcoal (0.3 g) as catalyst. The mixture was filtered. The intermediate amine (**11**) presumably obtained was not purified, due to instability. Formic acid (119 mg) was added and then dicyclohexyl carbodiimide (DCC) (356 mg) in dimethyl formamide (15 ml) was gradually added under N_2 to the soln of **11**, kept at 0°. The soln was first stirred at this temp for 1 hr and then the stirring was continued at room temp. overnight. The mixture was filtered, the filtrate was evaporated. The crude distamycin was purified by chromatography on a silica gel column (90 gr). The eluent used was $CHCl_3$:EtOH:water (100:50:4). On crystallization from EtOH-EtOAc distamycin-A hydrochloride (135 mg; 15%) was obtained, m.p. 189–193° (lit.[5] 186–9°); δ(DMSO-d_6) 2.7 (2 H, tr), 3.35 (2 H, tr), 3.82, 3.9, (3 N–CH_3), 6.97, 7.06, 7.22 (6 arom H), 8.15 (formyl H; aliphatic amide H); 8.89 (mult amidin H's), 9.86, 10.09 (arom. amide H's); λ_{max}^{EtOH} 240, 305 nm (ε, 25200, 30400); ν(KBr) 1650 cm^{-1}; *m/e* 464 (M^+–NH_3; 50%), 435 (7%), 410 (38%), 368 (14%), 273 (100%).; *m/e* (field desorption) 464, 409. A direct comparison (chromatographic behavior, m.p., spectral data) with natural distamycin showed complete identity.

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REFERENCES

[1] *Antiviral Drugs: Mode of Action and Chemotherapy of Viral Infections of Man* (Edited by Y. Becker) S. Karger, Basel (1976); C. E. Hoffmann, *Ann. Rept. Med. Chem.* **11**, 128 (1976).
[2] F. Arcamone, S. Penco, P. G. Orezzi, V. Nicollela and A. Pirelli; *Nature*, **203**, 1064 (1964); F. Arcamone, P. G. Orezzi, W. Barbieri, V. Nicolella and S. Penco, *Gazz. Chim. Ital.* **97**, 1097 (1967).
[3] P. Chandra, *Fortschr. Chem. Forsch.* **52**, 99 (1974); Y. Becker, *Isr. J. Med. Sci.* **11**, 1135 (1975); C. Zimmer, *Prog. Nucl. Acid. Res. Mol. Biol.* **15**. 285 (1975).
[4] F. Arcamone, S. Penco and F. Delle Monache, *Gazz. Chim. Ital.* **99**, 620 (1969); F. Arcamone, V. Nicolella, S. Penco and S. Radaelli, *Ibid.* **99**, 632 (1969); P. Chandra, S. Hausman and A. Metz, *Pharmacol. Res. Commun.* **6**, 311 (1974); S. L. Grokhovski, S. Zhuze and B. P. Gottikh, *Bioorg. Khem.* **1**, 1616 (1975).
[5] P. Penco, S. Redaelli and F. Arcamone, *Gazz. Chim. Ital.* **97**, 1110 (1967).
[6] P. Fournari, *Bull. Soc. Chim, Fr* 488 (1963); K. J. Morgan and D. P. Morrey, *Tetrahedron* **27**, 245 (1971).
[7] M. J. Weiss, J. S. Webb and M. Smith, Jr., *J. Am. Chem. Soc.* **79**, 1266 (1957).
[8] J. O. Thomas, *Tetrahedron Letters* 335 (1967).