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4,6-Didemethyl-4,6-dibromoactinomycin C_1 (D)

Frank Seela

Department of Chemistry, Yale University, New Haven, Connecticut 06520. *Received September 7, 1971*

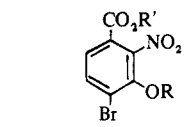
One varies the structure of the bacteriostatic and antitumor-active chromopeptide actinomycin C_1 for two reasons: improved therapeutic utility and elucidation of the inhibition mechanism. Microbiological modification of enlarged the group of actinomycins. This led to a better understanding of the inhibition mechanism, but did not result in a better therapeutic activity.

Kersten, *et al.*,[1] have shown that the antibiotic activity of actinomycin C_1 is based on its ability to inhibit mRNA synthesis by complex formation with DNA. A detailed model of complex formation was suggested by Müller and Crothers[2] and by Sobell, *et al.*[3]

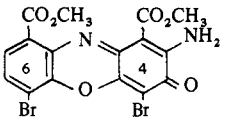
According to this model the chromophore of actinomycin and simple analogs[4] intercalates between the base pairs adjacent to guanine-cytosine. In agreement with the predictions based on this idea, the replacement of the 4,6 methyl groups by bulkier ethyl groups decreases the complex stability and the antibiotic activity; *tert*-butyl groups cause the activity to disappear.[5]

Replacement of the 4,6 methyl groups in actinomycin C_1 may also alter the electron distribution of the phenoxazone moiety and its electronic interactions with the guanine-cytosine base pair. In order to study the influence of substituent electronegativity on antibacterial activity and DNA complex stability in the absence of any major steric change and to improve our knowledge of the inhibition mechanism, the bromo analog 8 was synthesized. This compound is the first analog which has 4,6 substituents (bromine) with approximately the same van der Waals radii (r_{Br} = 1.95 Å, r_{CH_3} = 2.00 Å) as the methyl groups of actinomycin C_1, but with a higher electronegativity. Furthermore the heavy atoms should make it useful for crystallographic studies of the interaction with DNA.

The synthesis followed essentially a strategy which was successfully employed previously in synthesizing actinomycins[6] and actinomycin derivatives.[5] Nitration and esterification of 4-bromo-3-hydroxybenzoic acid[7,8] gave compound 1c. Esterification was necessary in order to separate an isomeric side product of the nitration by crystallization. Saponification of the ester 1b gave the acid 1a. Treatment of the disodium salt of 1a with excess benzyl chloride formed the benzyl ester-benzyl ether of 1a, which was saponified to yield 1c. *Via* the acid chloride, 1c was coupled with L-threonine to compound 3. Condensation of 3 and the peptide 4 with Woodward's reagent[9] gave 5a, debenzylation of 5a to 5b was accomplished with hydrobromic acid in glacial acetic acid. Sodium hydroxide hydrolyzed 5b to 5c. By sodium dithionite reduction, 5c was converted to 6 which was not isolated because of its air sensitivity, but was oxidized immediately to 7 with potassium ferricyanide. Ring closure of crude 7 with acetylchloride–acetylimidazole[10] formed 8.



1a, R = H, R' = H
1b, R = H, R' = CH_3
1c, R = CH_2Ph, R' = H



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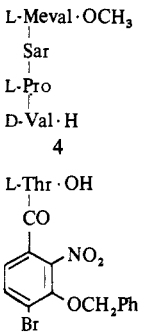

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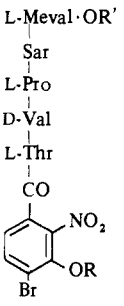
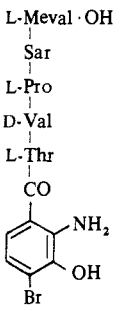


5a, R = CH_2Ph, R' = CH_3
5b, R = H, R' = CH_3
5c, R = H, R' = H



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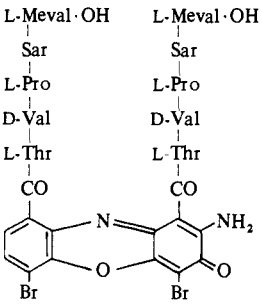

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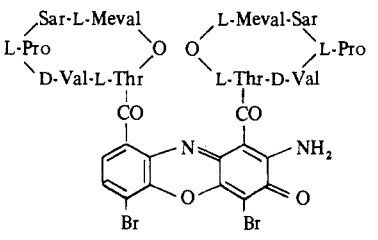

8

The main problem of the synthesis was the lability of the bromine substituents toward catalytic hydrogenation, a reaction which was used for removal of the benzyl protecting groups, and the conversion of the nitro to an amino group.[5] Catalytic hydrogenation of 1b followed by oxidative condensation with potassium ferricyanide, for example, did not result in 2. Instead, 2-amino-3*H*-phenoxazin-3-one-1,9-dicarboxylic acid methyl ester[11] was formed by loss of halogen. Hydrogenation was avoided by use of sodium dithionite for reduction of the nitro group. Using this method the compounds 2 and 7 were obtained with retention of bromine. Cleavage of the benzyl group was carried out with hydrobromic acid in glacial acetic acid.

Microbiological Testing. For antibacterial tests *Bacillus subtilis* (ATCC 6633) was used as a test organism. The method is described elsewhere.[12] The minimal concentration for the complete inhibition of the growth of *Bacillus subtilis* was 0.31 μg/ml for the actinomycin analog 8 and 0.07 μg/ml for actinomycin C_1 (standard).

This indicates that not only a larger size[5] but also a higher electronegativity of the 4,6 substituents decreases the antibacterial activity of actinomycin C_1.

Experimental Section

Melting points were determined with a Reichert apparatus and are corrected. Microanalyses were performed by Mikroanalytisches Laboratorium, Alfred Bernhardt, Germany. Where analysis is indicated by symbols of element, analytical results obtained for elements

were within ±0.4% of the calculated value. The nmr spectra were measured at 100 MHz on a Jeolco minimar-100 spectrometer. The λ_{max} and ϵ values were obtained with a Cary 14 spectrometer. Cellulose powder (Schleicher and Schuell, grade 286) was used for the cellulose column chromatography and the sodium *m*-cresotate solutions were saturated with *m*-cresotic acid.

Methyl 4-Bromo-2-nitro-3-hydroxybenzoate (1b). A stirred solution of 21.7 g of 4-bromo-3-hydroxybenzoic acid[9,10] in 500 ml of H_2SO_4 (*d* 1.84) was kept at 0–5° in an ice bath while a chilled mixture of 4.25 ml of fuming HNO_3 (90%) in 250 ml of H_2SO_4 (*d* 1.84) was added dropwise. The stirring was continued for an additional 0.5 hr and the resulting dark solution was poured into 3.0 kg of ice. After extraction with EtOAc and evaporation of the solvent the crude residue (25 g) was dissolved in 200 ml of MeOH and dry HCl was bubbled through the refluxing solution for 8 hr. Addition of H_2O to the cold solution precipitated an ester mixture (main and minor spot on tlc, $CHCl_3$); recrystallization (MeOH–H_2O) removed the minor compound and gave 16.3 g (59%) of light yellow plates of the pure ester **1b**; mp 124–126°; nmr ($CDCl_3$) δ 3.90 (s, 3), 7.09 (d, 1, *J* = 8 Hz), 7.84 (d, 1, *J* = 8 Hz), 9.80 (1). *Anal.* ($C_8H_6BrNO_5$) C, H, Br, N.

Dimethyl 2-Amino-4,6-dibromo-3*H*-phenoxazin-3-one 1,9-Dicarboxylate (2). $Na_2S_2O_4$ (2 g) was added gradually, with stirring, to a heated solution (50°) of 1.0 g of **1b** in 100 ml of 0.5 *N* $NaHCO_3$ until the color vanished. After the mixture had been cooled it was extracted with $CHCl_3$, and the organic layer was evaporated. The residue was dissolved by stirring it with 50 ml of MeOH diluted with warm phosphate buffer (100 ml, pH 7.2, 50°), and a solution of 4.0 g of $K_3[Fe(CN)_6]$ in 30 ml of phosphate buffer was added in several portions. The pH was always kept at 7.2 by the addition of diluted NaOH. After the red condensation product had stirred for 1 hr it was extracted with $CHCl_3$, the organic layer washed with water, and the solvent evaporated. Recrystallization of the residue (MeOH–$CHCl_3$) yielded 400 mg (45%) of red-brown needles; mp 216–218°; λ_{max} (MeOH) 427, 235 mμ (ϵ 28300, 24600); nmr ($CDCl_3$) δ 3.97 (s, 6), 7.60 (d, 1, *J* = 8 Hz), 7.72 (d, 1, *J* = 8 Hz), 7.70 (NH_2). *Anal.* ($C_{16}H_{10}Br_2N_2O_6$) C, H, Br, N.

4-Bromo-2-nitro-3-hydroxybenzoic Acid (1a). Compound **1a** (7.28 g) in 100 ml of 1 *N* NaOH was kept at 70° for 1.5 hr. After acidification of the cold solution, collection of the crystalline precipitate, and drying *in vacuo*, 6.4 g (93%) of light yellow plates was obtained. For the analytical data **1a** was recrystallized (MeOH–H_2O); mp 222°. *Anal.* ($C_7H_4BrNO_5$) C, H, Br, N.

4-Bromo-2-nitro-3-benzyloxybenzoic Acid (1c). KOH (2.5 g) in 25 ml of MeOH and 5.2 g of PhCHCl were added successively to a stirred solution of 5.11 g of **1b** in 10 ml of MeOH. After the mixture had refluxed with stirring for 10 hr, 3.3 g of NaOH in 20 ml of H_2O was added and stirred at its bp for 30 min. Steam distillation removed MeOH and $PhCH_2OH$. After the mixture had been cooled, **1c** was precipitated by acidification. After recrystallization (MeOH–H_2O) 4.9 g (71%) of colorless prisms, mp 175–176°, was obtained. *Anal.* ($C_{14}H_{10}BrNO_5$) C, H, Br, N.

***N*-(4-Bromo-2-nitro-3-hydroxybenzoyl)-L-threonin (3).** A suspension of 3.58 g of **1c** in 10 ml of $SOCl_2$ was refluxed for 10 min and the solvent removed under reduced pressure. The dry crystallized acid chloride in 40 ml of Et_2O–PhH (1:1) was combined with a solution of 1.2 g of L-threonine in 20.5 ml of 0.5 *N* NaOH at 0–5° and stirred at high speed. Dropwise addition of 10 ml of 1 *N* NaOH during a period of 30 min, followed by stirring for 3 hr (0–5°), led to emulsification. The layers were separated by centrifugation. The water layer was washed with Et_2O and acidified, and the reaction product extracted with EtOAc. From a reduced volume of the EtOAc solution 3.3 g (53%) of colorless crystals was precipitated by addition of benzene. For the analytical data the sample was recrystallized from EtOAc–C_6H_{12}; mp 192–193°; $[\alpha]^{20}D$ −52° ± 2° (*c* 1.5, ethanol). *Anal.* ($C_{18}H_{17}BrN_2O_7$) C, H, Br, N.

***N*-(4-Bromo-2-nitro-3-benzyloxybenzoyl)-L-threonyl-D-valyl-L-prolylsarcosyl-L-*N*-methylvalinemethyl Ester (5a).** Compound **3** (1.22 g) in 10 ml of $MeNO_2$ was neutralized with 0.375 ml of $(Et)_3N$ and then 0.7 g of powdered *N*-ethyl-5-phenylisoxazolium-3′-sulfonate[10] was added in portions. After the mixture had been stirred for 8 min, a solution containing 1.21 g of D-valyl-L-propylsarcosyl-*N*-methyl-L-valinemethyl ester hydrochloride and 0.375 ml of triethylamine in 15 ml of nitromethane was added. The mixture was stirred for 15 hr at room temp and the solvent evaporated. The dissolved residue ($CHCl_3$) was washed with 1 *N* HCl and H_2O, the layers were separated by centrifugation, and the solvent was evaporated. The dissolved residue (PhH) was adsorbed on an alumina column (Merck AG, activity II–III), washed with PhH, and eluted with $CHCl_3$. After filtration and evaporation 1.15 g (55%) of colorless powder was obtained (tlc, $CHCl_3$–MeOH, 95:5, one spot), $[\alpha]^{19}D$ −53° ± 2° (*c* 0.85, MeOH). *Anal.* ($C_{38}H_{51}BrN_6O_{11}$) C, H, Br, N.

***N*-(4-Bromo-2-nitro-3-hydroxybenzoyl)-L-threonyl-D-valyl-L-prolylsarcosyl-*N*-methyl-L-valinemethyl Ester (5b).** Compd **5a** (1.75 g) was dissolved in 30 ml of a 5 *N* solution of dry HBr in glacial AcOH and was stirred at room temp for 100 min. The reaction vessel was protected from atmospheric moisture with a $CaCl_2$ drying tube. The solution was diluted with a 10-fold excess of $CHCl_3$ and washed several times with H_2O in order to remove HBr and AcOH. Addition of cyclohexane to the reduced volume of the organic layer precipitated **5b**, which gave 1.4 g (90%) of cream-colored powder after centrifugation and drying *in vacuo*; one spot on tlc ($CHCl_3$–MeOH, 9:1), slower migrating than **5a** $[\alpha]^{25}D$ −28° ± 2° (*c* 0.8, MeOH). *Anal.* ($C_{31}H_{45}BrN_6O_{11}$) C, H, Br, N.

***N*-(4-Bromo-2-nitro-3-hydroxybenzoyl)-L-threonyl-D-valyl-L-prolylsarcosyl-*N*-methyl-L-valine (5c).** NaOH (5 ml, 1 *N*) was added to a solution of 1.27 g of **5b** in 1.7 ml of MeOH. The mixture was stirred for 1 hr at 40°, diluted with 200 ml of H_2O, and washed with $CHCl_3$. Acidification, extraction with $CHCl_3$, filtration, and evaporation gave 1.15 g (93%) of a yellow powder; $[\alpha]^{23}D$ −72° ± 3° (*c* 1.0, MeOH). *Anal.* ($C_{30}H_{43}BrN_6O_{11}$) C, H, Br, N.

4,6-Dimethyl-4,6-dibromoactinomycin C_1 Acid (7). A solution of 552 mg of **5c** in 10 ml of MeOH and 30 ml of phosphate buffer (pH 7.2) was treated with small portions of 440 mg of $Na_2S_2O_4$ at 50° while stirring. (Excess of $Na_2S_2O_4$ can be detected with Methylene Blue paper.) After 15 min a solution of 1.2 g of $K_3[Fe(CN)_6]$ in 15 ml of phosphate buffer (pH 7.2) was added gradually (a constant pH of 7.2 was maintained by addition of small amounts of 1 *N* NaOH). After 1 hr the diluted (H_2O) and washed ($CHCl_3$) solution was acidified and extracted with a mixture of $CHCl_3$–BuOH (4:1). The washed (H_2O) organic layer was evaporated and the residue dissolved in MeOH. EtOAc precipitated amorphous **7** (265 mg, 50%, after drying) which was used without further purification to prepare **8**. For the analytical data a sample was purified by cellulose column chromatography (BuOH–BuAc–3% aqueous sodium *m*-cresotate, 7:3:10, v/v). After separation as described above, the cellulose was extruded, the slowly migrating zone cut out and suspended in MeOH, the cellulose filtered, and the filtrate diluted with H_2O. Acidification (2 *N* HCl) and extraction with $CHCl_3$ followed by evaporation gave a product which was adsorbed on a silica gel column (acidic silica gel) and washed with $CHCl_3$ to remove *m*-cresotic acid. Elution with MeOH, dilution with H_2O, and acidification (2 *N* HCl) gave **7** which could be extracted with $CHCl_3$. After evaporation, the residue was dissolved in MeOH and **7** precipitated with EtOAc; yellow-red powder; mp 195–198°; λ_{max} (MeOH) 447, 425, 242 (ϵ 25500, 23600, 39100); $[\alpha]^{23}D$ −138° ± 5° (*c* 0.9, MeOH). *Anal.* ($C_{60}H_{84}Br_2N_{12}O_{18}$) C, H, Br, N.

4,6-Didemethyl-4,6-dibromoactinomycin C_1 (8). The stirred solution of 388 mg of **7** in 23 ml of THF was treated with 128 ml of warm cyclization reagent[11] for 2 hr at 55°. After evaporation, the residue was dissolved in $CHCl_3$ and thoroughly washed with 2 *N* HCl, saturated $NaHCO_3$, and H_2O, and the organic layer evaporated. The dissolved red-brown product was adsorbed on an alumina column (15 × 2 cm, Merck AG, activity IV, PhH) washed with PhH and eluted with EtOAc. After evaporation minor impurities were removed on a cellulose column (40 × 4 cm, BuOH–Bu_2O–10% aqueous sodium *m*-cresotate, 2:3:5). The separated main zone was cut out after extrusion of the cellulose, the material suspended in MeOH, the cellulose filtered, and the filtrate diluted with H_2O. The PhH extract of the filtrate was washed with saturated $NaHCO_3$, 2 *N* HCl, and H_2O, and the organic solvent evaporated. Adsorption of the residue on an alumina column (10 × 2 cm, Merck AG, activity IV, PhH) and elution with EtOAc gave a solution of **8** after filtration. After evaporation and recrystallization (EtOAc–C_6H_{12}), 46 mg (12%) of yellow-red bipyramids was obtained; mp 255–257°; λ_{max} (MeOH) 444, 424, 246 mμ (ϵ 24900, 23900, 32600); $[\alpha]^{23}D$ −250° ± 10° (*c* 0.16, MeOH). *Anal.* ($C_{60}H_{80}Br_2N_{12}$) C, H, N. On a paper chromatogram (butyl acetate–10% aqueous sodium *m*-cresotate, 1:1) the R_f value of **8** is 1.5 times as large as that of actinomycin C_1 (D).

Acknowledgment. I wish to thank Dr. D. M. Crothers for his support of this work through a grant (T-523) from the American Cancer Society.

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Studies on the Syntheses of Heterocyclic Compounds. 459.[1] Synthesis of Rescinnamine-Like Compounds as Antihypertensive Agents

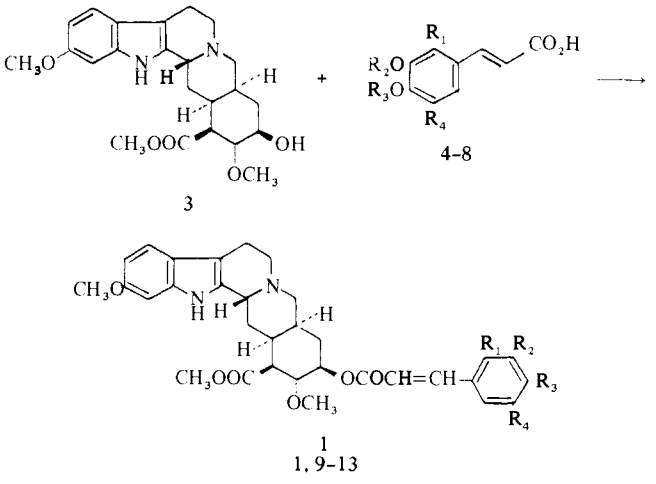
T. Kametani,* M. Ihara, T. Suzuki, T. Takahashi, R. Iwaki, H. Takei, N. Miyake, M. Yoshida, Y. Hasegawa, and H. Kitagawa

Pharmaceutical Institute, Tohoku University, Aobayama, Sendai, Japan, Research Laboratories, Nippon Chemipha Co., Ltd., Honkomagome, Bunkyo-ku, Japan, and Department of Pharmaceutical Science, Chiba University, Chiba, Japan.
Received November 9, 1971

Although rescinnamine (**1**) and reserpine (**2**) have essentially equal pharmacological activity the side effects of rescinnamine are weaker than those of reserpine. In hope of finding more pronounced biological activity we have synthesized some new derivatives which have a cinnamoyl substituent[2] at the 18 position of methyl reserpate (**3**).

Methyl reserpate (**3**)[3] was esterified with acid chlorides derived from 3,4-dimethoxycinnamic acid (**4**),[4] 4-ethoxy-3-methoxycinnamic acid (**5**),[5] 3,4,5-trimethoxy-2-nitrocinnamic acid (**6**),[6] 3-ethoxycarbonyl-4-methoxycinnamic acid (**7**),[7] and 4-ethoxycarbonyl-3-methoxycinnamic acid (**8**)[8] in pyridine–PhH to give rescinnamine-like derivatives **9**, **10**, **11**, **12**, and **13**, respectively. Treatment of **12** and

Scheme I



13 with 1 equiv of NaOH in a soln of MeOH and THF at room temp gave the phenolic bases **14** and **15**.

*Author to whom correspondence should be addressed at Tohoku University, Aobayama, Sendai, Japan.

Table I. Synthesis (Scheme I) and Antihypertensive Activity of Rescinnamine-Like Compounds

Starting materials							Products								Antihypertensive activity	
Compd	Amt, g	Compd	Amt, g	R_1	R_2	R_3	R_4	Compd	R_1	R_2	R_3	R_4	Yield, %	Mp, °C	Solv used for recrystn	Formula[a]	ED_{20}, mg/kg	Relative activity calcd by ED_{20} (Reserpine = 1.00)
3	1	**4**[b]	2	H	CH_3	CH_3	H	**9**	H	OCH_3	OCH_3	H	55 (0.81 g)	180–181	MeOH–$CHCl_3$	$C_{34}H_{40}N_2O_8$	1.0	1.40
3	0.5	**5**[c]	1.5	H	CH_3	C_2H_5	H	**10**	H	OCH_3	OC_2H_5	H	75 (0.56 g)	140–141	$CHCl_3$–hexane	$C_{35}H_{42}N_2O_8$	1.6	0.86
3	1.2	**6**[d]	2	NO_2	CH_3	CH_3	OCH_3	**11**	NO_2	OCH_3	OCH_3	OCH_3	87 (1.52 g)	Picrate 147–150	MeOH	$C_{41}H_{44}N_6O_{18}$	1.0	1.40
3	1.3	**7**[e]	2	H	$CO_2C_2H_5$	CH_3	H	**12**	H	$OCOC_2H_5$	OCH_3	H	75 (1.6 g)	Styphnate 200–201	MeOH	$C_{42}H_{45}N_5O_{18}$	2.5	0.56
3	1.5	**8**[f]	3	H	CH_3	$CO_2C_2H_5$	H	**13**	H	OCH_3	$OCOC_2H_5$	H	74 (1.8 g)	Picrate 160–161	MeOH	$C_{42}H_{45}N_5O_{17}$	1.5	0.94
								1 Reserpine (**2**)	H	OCH_3	OCH_3	OCH_3		238–239 dec 264–265 dec			1.4 0.2	1.00 7.00

[a] All compds were analyzed for C, H, N. Ir and nmr spectra were as expected. [b] See ref 4. [c] See ref 5. [d] See ref 6. [e] See ref 7. [f] See ref 8.