tions of the signs of J_{C-H} were tried.[7] Of the four possible relative sign combinations for $J_{C_7-H_2}$, $J_{C_7-H_3}$, and $J_{C_7-H_4}$—[+++], [−++], [+−+], and [++−][8]—it quickly became apparent from the simulated spectra that the last two cases could be ruled out. Of the remaining two possibilities, the relative sign combination [+++] was favored because a smaller root-mean-square error could be obtained (0.039 Hz for [+++] *vs.* 0.08 Hz for [−++]). This tentative conclusion was substantiated by a close look at weak combination lines. The region between 785 and 795 Hz included four signals which by the LAOCOON study were shown to be particularly sensitive to the relative signs of the J_{C-H} values. Comparison of these observed frequencies with those frequencies predicted by the various relative sign combinations indicated the [+++] combination to be the best.[9] Figure 1 compares observed and simulated spectra for methyl benzoate-*carboxyl*-^{13}C using the [+++] combination.

To confirm the [+++] relative spin combination, spin-tickling experiments were conducted.[10] Three different frequencies were irradiated and in each case collapse of various signals unequivocally showed the relative sign combination (+++) to be correct and the relative sign combination (−++) to be incorrect.[11]

Although it was not possible in the LAOCOON study to decide between the absolute sign combinations [+++] and [−−−], it was hoped that the spin-tickling experiment would do this. Disappointingly, virtually identical collapsing patterns were predicted to result from both absolute sign combinations [+++] and [−−−]. However, in one instance a choice could be made, and the results clearly favored [+++]. This conclusion, albeit tentative, is consistent with previous absolute J_{C-H} sign determinations; the sign of $^3J_{C-H}$ is positive in acetone,[1r] and the sign of $^3J_{C-H}$ is positive (but that for $^4J_{C-H}$ is negative) in benzene[1b] and in dihalobenzenes.[10] Furthermore, if one attempts to relate the J_{C-H} values in this present study to the J_{H-H} values in the model compound[12] benzene, the correspondence is quite good,[13] lending substantiation to the contention that the J_{C-H} signs in the benzoate system **1** are all positive as for the J_{H-H} signs in the geometrically equivalent benzene molecule **2**.

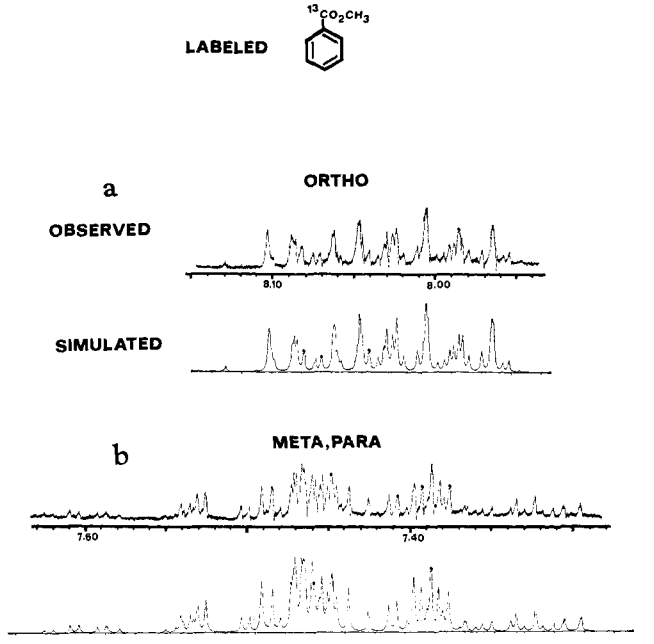
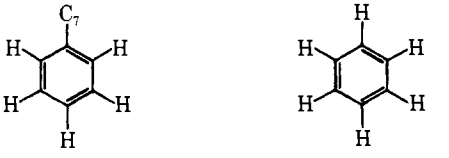


Figure 1. Observed and simulated pmr patterns for methyl benzoate-*carboxyl*-^{13}C: (a) ortho region; (b) meta, para region.



1 2

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(14) Robert A. Welch Postdoctoral Fellow, 1970–1972.

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(7) The J_{H-H} signs were all held positive [J. M. Emsley, J. Feeney, and L. H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Vol. II, Pergamon Press, Elmsford, N. Y., 1966, pp 682, 771].

(8) In this LAOCOON study it was not possible to differentiate between the absolute sign combinations, *e.g.*, the predicted spectrum for the sign combination [+++] was identical with that for the combination [−−−].

(9) The experimental values of these low-intensity frequencies and those predicted by the various sign combinations were

Rel sign combination, $J_{C_7-H_o}$, $J_{C_7-H_m}$, $J_{C_7-H_p}$	Transition frequencies, Hz				Av error
Exptl	787.87	790.13	792.37	794.67	
(+++)	787.83	790.17	792.32	794.70	0.04
(+−+)	788.60	791.07	791.48	793.85	0.84
(++−)	787.43	789.78	792.65	795.11	0.38
(−++)	788.19	790.63	791.86	794.27	0.43

(10) S. M. Castellano and A. A. Bothner-By, *J. Chem. Phys.*, 47, 5443 (1967).

(11) These spin-tickling experiments also confirmed that the previously discarded relative sign combinations [+−+] and [++−] were incorrect.

(12) For an example of relating J_{H-H} values in model compounds to J_{C-H} values in geometrically equivalent systems, see ref 1b.

(13) The $^3J_{H-H}$, $^4J_{H-H}$, and $^5J_{H-H}$ values of benzene, respectively, 7.7, 1.4, and 0.6 Hz [S. Castellano and C. Sun, *J. Amer. Chem. Soc.*, **88**, 4741 (1966)], compare with benzoate $^3J_{C-H}$, $^4J_{C-H}$, and $^5J_{C-H}$ values, respectively, 4.1, 1.1, and 0.5 Hz.

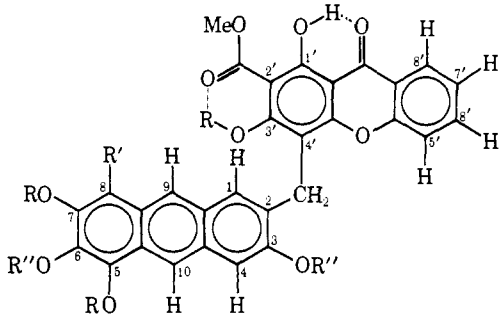
Structure of Thermorubin A, the Major Orange-Red Antibiotic of *Thermoactinomyces antibioticus*

Sir:

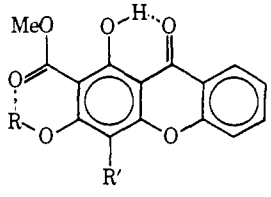
Growth of *Thermoactinomyces antibioticus*,[1] a thermophilic actinomycete, in submerged culture produces a mixture of orange-red antibiotics which are highly active against both Gram-positive and Gram-negative bacteria; the major component is thermorubin A.[2] Structure I deduced (*vide infra*) for this antibiotic is unique by virtue of the presence, within the same molecular framework, of xanthone and anthracene moieties which presumably arise from the same polyketide precursor.

(1) R. Craveri, C. Coronelli, H. Pagani, and P. Sensi, *Clin. Med.*, 71, 511 (1964).

(2) Thermorubin A is identical with the antibiotic BT 3-3 described by M. Terao, K. Furuya, and R. Enokita, *Sankyo Kenkyusho Nempo*, 17, 110 (1965).



I, R = R′ = H; R″ = Me
II, R′ = H; R = R″ = Me
VI, substitution within anthracene moiety 5 × H, 4 × OMe
VII, R = CH_nD_{3-n}; R′ = D; R″ = Me



III, R = H; R′ = $C_{17}H_{15}O_4$
IV, R = Me; R′ = $C_{19}H_{19}O_4$
V, R = Me; R′ = H

There are several cases on record of the cooccurrence of structurally, closely related anthraquinones and xanthones. Thus, *Aspergillus versicolor* (Vuill.) Tiraboschi gives rise to the anthraquinones averufin[3-5] and aversin,[6] together with the xanthones sterigmatocystin[3] and 6-methoxysterigmatocystin,[6] while the pigments from *Claviceps purpurea* contain the anthraquinone endocrocin together with the complex xanthone derivatives ergoflavin and secalonic acid A.[7] Thermorubin A (I), however, is the first example of a metabolite possessing both a xanthone and a modified anthraquinone moiety.

Thermorubin A (I)[8] [$C_{32}H_{24}O_{10}\cdot 2H_2O$; ir (Nujol mull) 3400–2200, 1705, 1610, and 1570 cm^{-1}; λ_{max}^{EtOH} 250, 300, 328, 415 (shoulder), and 435 nm (ϵ 33,700, 54,640, 49,980, 15,100, and 16,300); $\lambda_{max}^{EtOH-NaOAc}$ 328 → 335 nm; mp >200° dec[10]] crystallizes as orange-red rosettes from ethyl acetate and analyzes for three methoxyl groupings. The intense peaks at 1610 and 1570 cm^{-1} suggested a 1-hydroxyxanthone and this was substantiated by a positive Dimroth test.[11,12]

Treatment of I with 0.1 *N* sodium hydroxide led to a complex mixture[13] which displays intense, but broad absorption in the infrared spectrum (3700–2200 cm^{-1}) indicative of a carboxylic acid grouping. Methylation of this mixture or of thermorubin A[13] itself with diazomethane led to trimethylthermorubin A (II): mp 121–123°; ir (Nujol mull) 3200–2100, 1740, 1630, and 1600 cm^{-1}; λ_{max}^{EtOH} 253, 296, 325,[14] 403 (shoulder), and 424 nm (ϵ 27,000, 39,200, 50,800, 6,500, and 7,900). Displacement of the ester carbonyl from 1705 to 1740 cm^{-1} on methylation indicated intramolecular hydrogen bonding of the carbomethoxyl of I.

Fusion of I with caustic soda[13] resulted in selective detachment of the carbonyl of the xanthone from the more reactive phenol[15] to give salicylic acid.

The base peak *m/e* 610 of the mass spectrum of II proved to be the molecular ion. Ring fission *via* a retro-Diels–Alder reaction is responsible for the intense *m/e* 120 ($C_7H_4O_2$) and 121 ($C_7H_5O_2$) peaks. A principal mode of fragmentation of II is the loss of methanol to give a peak at *m/e* 578. This type of decomposition is common to salicylates.[16,17] Loss of the methoxyl radical (α cleavage) is responsible for the peak at *m/e* 579. The further fragmentations of these two species, *i.e.*, *m/e* 578 and 579, are unspectacular and involve consecutive expulsions of carbon monoxide and methyl radical.

The data presented thus far permit us to write III and IV as part structures for thermorubin A and trimethylthermorubin A, respectively.

Corroboration for these views was derived by synthesis of 1-hydroxy-2-carbomethoxy-3-methoxyxanthone (V) [mp 179–181°; ir (Nujol mull) 3200–2200, 1740, 1655, and 1610 cm^{-1}; λ_{max}^{EtOH} 246, 300, and 340 nm (ϵ 38,500, 16,020, and 7,080)] from 1,3-dihydroxyxanthone by carboxylation with Stiles' reagent[18] and subsequent methylation with diazomethane.

The high-resolution mass spectrum of V proved most informative since it possesses many of the features common to trimethylthermorubin A (II). The retro-Diels–Alder fragmentation characteristic of the γ-pyrone system is very significant, giving intense peaks at *m/e* 120 and 121. Metastable transitions, detected by the defocusing technique, demonstrate the further fragmentation of the species *m/e* 268 (M − MeOH) and 269 (M − OMe) by consecutive losses of carbon monoxide and methyl radical.

Figures 1 and 2 are expanded spectra of the aromatic and methoxyl regions of II in deuteriobenzene.[19] These reveal six methoxyls, a methylene group of the diphenylmethane type, and nine aromatic protons, consisting of five "singlets" and the four-spin system of the xanthone moiety. In conjunction with the part structure IV only an anthracenyl or a phenanthrenyl nucleus permits accommodation of the functionality referred to above. The correctness of a β-methylanthracene moiety leading to part structure VI for trimethylthermorubin A, and the assignment of the remaining substituents, *i.e.*, the four methoxyl and the

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(6) E. Bullock, D. Kirkaldy, J. C. Roberts, and J. G. Underwood, *J. Chem. Soc.*, 829 (1963).
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(8) Satisfactory elemental analyses (±0.3%) were obtained for all new compounds.
(9) K. R. Markham, *Tetrahedron*, **21**, 3687 (1965).
(10) The antibiotic is thermally labile, decomposing rapidly in solution at temperatures greater than *ca.* 60°.
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(12) L. F. Fieser, *J. Amer. Chem. Soc.*, **51**, 2471 (1929).
(13) Personal communication from Dr. N. Maggi, Research Laboratories of Gruppo Lepetit, Milan, Italy.
(14) There is no displacement of the 325-nm band on addition of sodium acetate in this case.
(15) H. Raistrick, R. Robinson, and D. E. White, *Biochem. J.*, **30**, 1303 (1936).
(16) F. W. McLafferty and R. S. Gohlke, *Anal. Chem.*, **31**, 2076 (1959).
(17) E. M. Emery, *ibid.*, **32**, 1495 (1960).
(18) M. Stiles, *J. Amer. Chem. Soc.*, **81**, 2598 (1959).
(19) To minimize viscosity effects and to maximize chemical-shift differences trimethylthermorubin A (II) was studied in C_6D_6 at +88°.

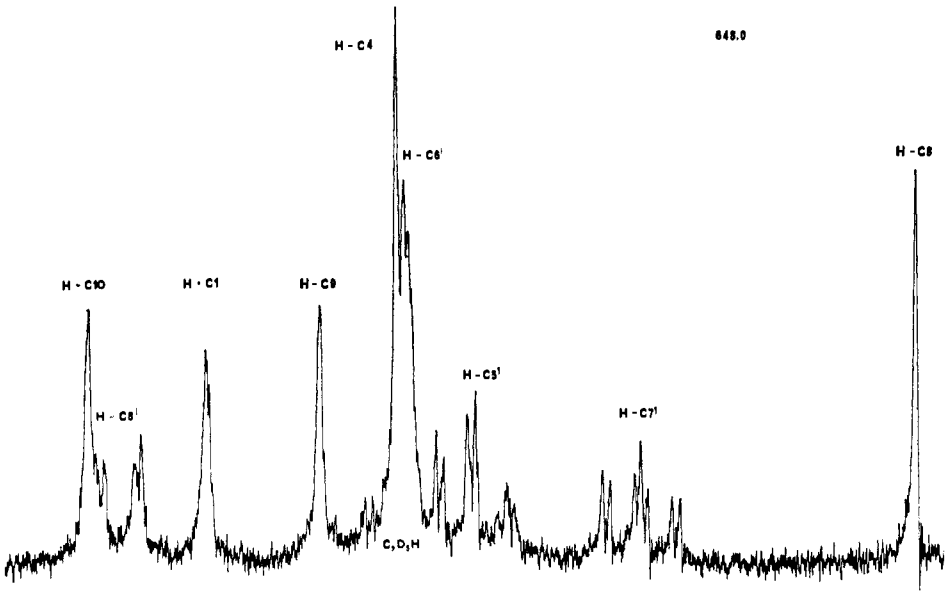

Figure 1. Expanded aromatic region of trimethylthermorubin A in C_6D_6; sweep width, 250 Hz.

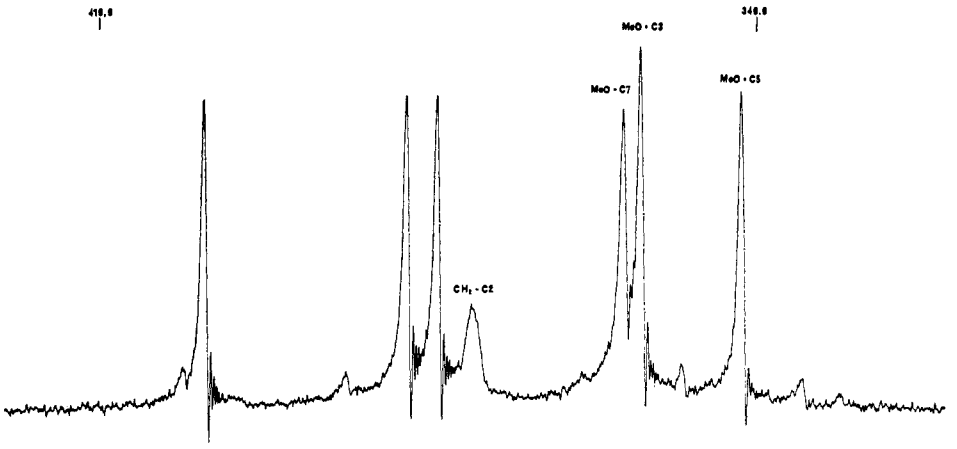

Figure 2. Expanded methoxyl region of trimethylthermorubin A in C_6D_6; sweep width, 100 Hz.

five aromatic protons, follows quite clearly from the nmr data.

Selective decoupling experiments in conjunction with a theoretical analysis permitted assignment of the AMNX (= H-8′, H-6′, H-5′, H-7′) spin system of the xanthone moiety. The pertinent parameters are: $J_{H\text{-}8'\text{–}H\text{-}7'} = 8.25$, $J_{H\text{-}8'\text{–}H\text{-}6'} = 1.50$, $J_{H\text{-}8'\text{–}H\text{-}5'} = 0.45$, $J_{H\text{-}7'\text{–}H\text{-}6'} = 6.25$, $J_{H\text{-}7'\text{–}H\text{-}5'} = 1.70$, and $J_{H\text{-}6'\text{–}H\text{-}5'}$

Table I. Nmr Data for Trimethylthermorubin A (II)[a]

	Chemical shifts, ppm		Coupling constants, Hz							Nuclear Overhauser effects[d] (proton(s) irradiated), %		
	C_6D_6 (88°)	$CDCl_3$ (28°)	H C-4	H C-8	H C-9	H C-10	CH_2	MeO C-3	MeO C-7	(CH_2)	(MeO C-5)	(MeO C-7)
CO_2Me C-2′	*b*	*b*										
MeO C-3′	4.052	*b*										
MeO C-3	3.586	3.820										
MeO C-5	3.479	3.700										
MeO C-6	*b*	*b*										
MeO C-7	3.604	3.930										
CH_2 C-2	3.763	3.900										
H C-1	7.589	7.814	0.17[c]		0.10[c]	0.59	0.68			4.1		
H C-4	7.187	7.419			0.46	0.10[c]	0.30	0.22				
H C-8	6.089	6.254			0.21[c]	0.45			0.29			6.4
H C-9	7.347	8.257				0.38						
H C-10	7.840	7.861									21.8	

[a] Reference 20. [b] Could not be assigned; δ 3.800 and 3.836 (C_6D_6); δ 4.042, 4.049, and 4.160 ($CDCl_3$). [c] Upper limit values. [d] Given in per cent increase in intensity of observed proton when signal in parentheses is saturated.

= 7.65 Hz; δH-8′ = 7.788, δH-7′ = 6.675, δH-6′ = 7.182, and δH-5′ = 6.955.[20] The J values are in agreement with previously analyzed xanthone systems.[21]

In deuteriochloroform the two methoxyl peaks at δ 3.930 and 3.820 show anomalous, temperature-dependent, line-width behavior relative to the other methoxyl absorptions. These and the methylene group provide a subtle probe for assessing the locations of the anthracene ring protons.[22-24] Double irradiation of MeO C-3 and MeO C-7 causes a small line-width reduction and/or enhanced resolution of the splitting patterns for H-4 and H-8, respectively.[25,26] Similarly, irradiation of the methylene absorption causes H-1 to collapse to a broad doublet and H-4 to show enhanced resolution. Nuclear Overhauser effects (NOE)[20,27] were observed for H-8 and H-1 upon decoupling MeO C-7 and CH_2 C-2, respectively. In addition, saturation of MeO C-5 caused an integral enhancement for H-10.

The details of the spin–spin couplings between the anthracene protons were exposed by double and triple resonance experiments. For example, irradiation of H-10 brought about the collapse of H-1 to a broad triplet revealing $J_{CH_2-H\text{-}1}$. Adding a second oscillator tuned to CH_2–C-2 further collapsed H-1 to a singlet having a width at half-height (Δ) of 0.57 Hz.[28]

The results for II are summarized in Table I; they are consistent only with structure II. These assignments assume that $J_{epi} > J_{peri}$[29] and that only the methoxyl ortho proton coupling is detectable.[29]

Thermorubin A (I) itself is thermally labile,[10] thus precluding an exhaustive nmr study; however, its nmr behavior is very similar to trimethylthermorubin A (II) with one notable exception: the anthracene proton in II at highest field, H-8, is shifted downfield in I by 85.2 Hz (DMSO-d_6, C_6D_6). This effect may be attributed in part to the relief of steric crowding between the C-5, -6, and -7 methoxyl groups,[30] and in part to the effect of complexed dimethyl sulfoxide.[31]

Complete assignment of the structure of thermorubin A (I) was made possible by nmr examination of the deuterated analog of trimethylthermorubin A (VII) obtained by treatment of I with deuteriodiazomethane.[32] Three partially deuterated methoxyl groups (δ 3.48, 3.61, and 4.05, ≈50% D) were evident in the nmr (C_6D_6, 88°) of VII. By direct comparison of deuteriotrimethylthermorubin A (VII) with II the OCH_nD_{3-n} resonances at δ 3.48 and 3.61 can be assigned to the methoxyl groups at C-5 and C-7. The proton, H–C-8, undergoes complete deuterium exchange as would be anticipated for a *m*-dihydroxybenzene derivative. The remaining OCH_nD_{3-n} resonance can be assigned to the methoxyl group at C-3′. These facts together with the data presented earlier dictate that structure I be assigned to thermorubin A.

(20) A Digital Equipment Corporation PDP-12 computer was used for (i) the theoretical line-shape analysis, (ii) substantiation of the NOE values, and (iii) resolution enhancement to aid in the direct determination of some of the coupling constants of the anthracene moiety (see Table I).

(21) D. Barraclough, H. D. Locksley, F. Scheinmann, M. T. Magalhães, and O. R. Gottlieb, *J. Chem. Soc. B*, 603 (1970).

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(28) Where coupling constants could not be obtained directly *via* such multiple irradiation experiments[20] the upper limit of their magnitudes could be calculated from Δ in the fashion employed by K. D. Bartle, *et al.*[25]

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(32) K. J. van der Merwe, P. S. Steyn, and S. H. Eggers, *Tetrahedron Lett.*, **52**, 3923 (1964).

(33) Lepetit visiting scientist at the Eastern Research Laboratory, 1970–1971.

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Received December 2, 1971

Total Synthesis of Gougerotin[1]

Sir:

Gougerotin,[2] a nucleoside antibiotic isolated by Kanzaki, *et al.*,[3] from *Streptomyces gougerotii*, inhibits protein synthesis by preventing the transfer of amino acids from aminoacyl tRNA to polypeptide.[4] Gougerotin is also an inhibitor of the multiplication of certain viruses.[5] The structure of this antibiotic was established (see Scheme I) in our laboratory as 1-[4-deoxy-4-(sarcosyl-D-seryl)amino-β-D-glucopyranosyluronamide]-cytosine.[6] Recently the syntheses of methyl 4-amino-4-deoxy-α-D-glucopyranosiduronic acid[7] (a derivative of the carbohydrate moiety of Gougerotin) and of 1-(4-amino-4-deoxy-β-D-glucopyranosyluronic acid)cytosine[8] (C substance, the nucleoside moiety of Gougerotin) were reported. We now describe the first chemical synthesis of 1-[4-deoxy-4-(sarcosyl-D-seryl)amino-β-D-glucopyranosyluronamide]cytosine (**7**) and its identity with Gougerotin. We also report the synthesis and characterization of Seryl-C (**4**), a hydrolysis product of the antibiotic.

The C substance[8] was converted with methanolic hydrogen chloride to the ester **1** which was isolated from the reaction mixture as the crystalline dihydrochloride monohydrate,[9] mp 217–223° dec, $[\alpha]^{27}D$ −14° (*c* 1.0,

(1) (a) Nucleosides. LXXV. Synthetic Studies on Nucleoside Antibiotics. 9. (b) For a previous pertinent paper see K. A. Watanabe, E. A. Falco, and J. J. Fox, *J. Org. Chem.*, **37**, 1198 (1972).

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