= 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.

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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,

(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).

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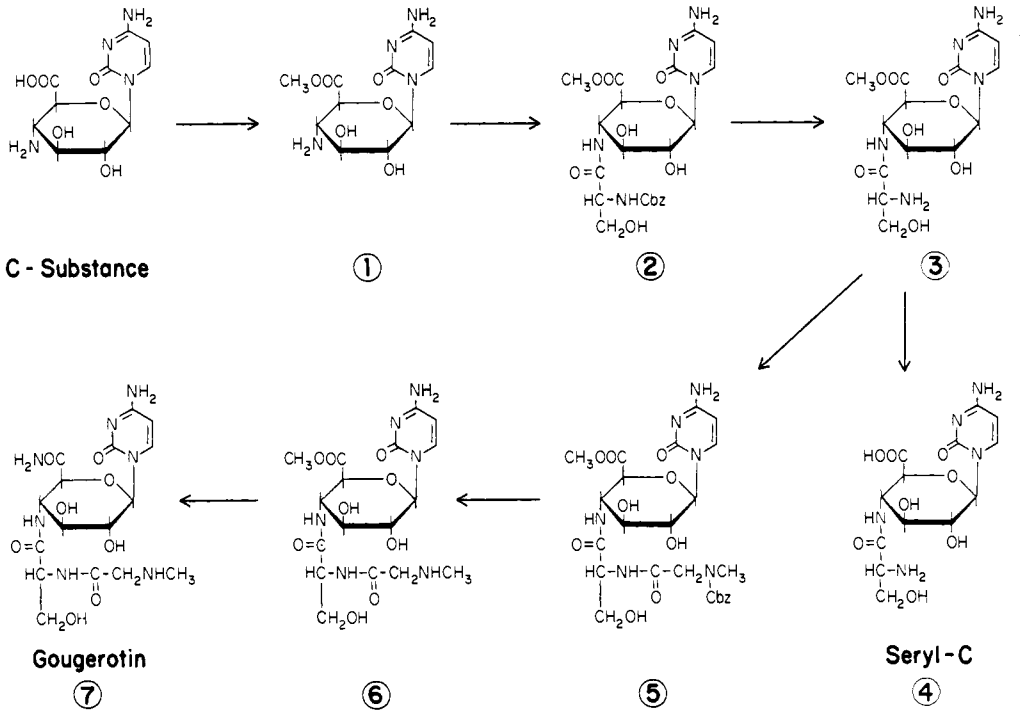
Scheme I



H_2O). Treatment of **1** with 2 equiv each of triethylamine, *N*-benzyloxycarbonyl-D-serine, and dicyclohexylcarbodiimide (DCC) in a 1:1 mixture of acetonitrile–methanol afforded **2** contaminated with the N_4,N_4'-diacylated derivative.[10] The crude product was treated briefly with an approximately 1:1 mixture of methanol-washed Dowex 50 (H^+) and Dowex 1 (OH^-) in absolute methanol at 0° and gave, after filtration and evaporation of solvent, a very high yield of compound **2**: mp 270–274° dec; $[\alpha]^{27}D$ +19° (*c* 0.7, MeOH); uv (in 0.1 *N* HCl in methanol) λ_{max} 277 nm (ϵ 10,500), λ_{min} 242 (2700); (in methanol) plateau at 257–265 (6700), λ_{max} 235 (8000), λ_{min} 228 (7800); (in 0.1 *N* NaOH in methanol) λ_{max} 267 (7100), λ_{min} 254 (6800); ir showed a band at 1730 cm^{-1} for ester.

Catalytic hydrogenolysis of the carbobenzoxy (Cbz) group of **2** over 10% Pd/C in 50% aqueous ethanol gave **3** which, after hydrolysis with dilute sodium hydroxide, yielded a crystalline product **4**: mp 230–235° dec; $[\alpha]^{27}D$ +57.3° (*c* 0.12, H_2O);[11] uv (in 0.1 *N* HCl) λ_{max} 274 nm (ϵ 11,400), λ_{min} 239 (2400); (in water) λ_{max} 266 and 232 (8000 and 7600), λ_{min} 252 and 225 (6900 and 7500); (in 0.1 *N* NaOH) λ_{max} 267 (8200), λ_{min} 251 (7200). The ir spectra (KBr disk) of compound **4** and of Seryl-C (obtained by Iwasaki by hydrolysis of Gougerotin in concentrated hydrochloric acid)[12] were identical. It should be noted that though hydrogenolysis of **2** afforded **3** as the sole product as evidenced by paper electrophoresis[13] (mobility of **3** = −2.4 cm), the presence of Seryl-C (mobility +0.3 cm) was observed after the evaporation of the solvent from the reaction mixture.

Hydrolysis of **3** to Seryl-C was avoided, however, by the addition of ~2 equiv of *N*-carbobenzoxysarcosine to the filtrate obtained after removal of catalyst from the hydrogenolysis reaction mixture before evaporation of solvent. The dried residue of **3** and *N*-carbobenzoxysarcosine was treated with DCC in an acetonitrile–methanol mixture. In this case again a small amount of N_4,N_4'-diacyl derivative was produced. After treatment of the crude product in a manner similar to that described for the preparation of **2**, colorless microcrystals of **5** were obtained in 80% yield: mp 189–194° efferv; $[\alpha]^{27}D$ +16° (*c* 1.2, MeOH); uv (in 0.1 *N* HCl in methanol) λ_{max} 277 nm (ϵ 13,600), λ_{min} 242 (3700); (in methanol) plateau at 257–265 (7200), λ_{max} 237 (8200), λ_{min} 228 (8000); (in 0.1 *N* NaOH in methanol) λ_{max} 267 (8400), λ_{min} 253 (7900); ir band at 1730 cm^{-1} for ester. The electrophoretic mobility was −1.5 cm.

After hydrogenolysis of compound **5** with Pd/C in aqueous ethanol, the filtered solution was diluted with 20 vol of ethanol and concentrated to dryness *in vacuo* below 30°. Compound **6** was obtained in quantitative yield as a white powder: mp 235–245° dec; $[\alpha]^{27}D$ +41° (*c* 0.6, H_2O); uv (in 0.1 *N* HCl) λ_{max} 274 nm (ϵ 11,200), λ_{min} 240 (2600); (in water) λ_{max} 266 and 237 (7700, 7900), λ_{min} 253 and 227 (7200 and 7500); (in 0.1 *N* NaOH) λ_{max} 268 (8500), λ_{min} 252 (7300); ir band at 1730 cm^{-1} for ester. The electrophoretic mobility was −3.4 cm. Compound **6** was treated with methanolic ammonia at room temperature overnight. After removal of solvent, the solid residue was dissolved in a minimal amount of water and diluted with a large volume of methanol. Compound **7** separated as fine needles (80% yield): mp 211–217° dec, efferv; $[\alpha]^{27}D$

(9) Satisfactory elemental analyses were obtained for all new compounds with melting points reported herein.

(10) This phenomenon of di-N,N''-acylation has been observed previously[1b] in the synthesis of 1-[4-deoxy-4-(sarcosyl-D-seryl)amino-β-D-glucopyranosyl]cytosine.

(11) Compound 4 (Seryl-C) is only sparingly soluble in water, hence the concentration of compound was low. We are indebted to Dr. David Fukushima of the Institute for Steroid Research, Bronx, N. Y., for the measurement of this rotation on a Rudolf polarimeter with a photomultiplier attachment.

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(13) Paper electrophoretic data were obtained in pH 7 phosphate buffer at 900 V for 2 hr.

$+53°$ (c 0.8, H_2O) [lit.[3] mp 210–215° dec; $[\alpha]^{21}D$ $+45°$]. A sample of Gougerotin kindly provided by Iwaski showed $[\alpha]^{27}D$ $+53°$. The uv data found for synthetic and natural Gougerotin were identical [uv in 0.1 N HCl) λ_{max} 275 nm (ϵ 13,600), λ_{min} 239 (3000); (in water) λ_{max} 267 and 235 (9400, 9300), λ_{min} 252, 227 (8500, 9200); in 0.1 N NaOH) λ_{max} 267 (9800), λ_{min} 252 (8900)]. The ir spectra of natural and synthetic Gougerotin were identical as were also their paper electrophoretic mobilities (−3.3 cm).

For biochemical and biological comparison, the natural and synthetic samples were chromatographed[14] (Whatman No. 1 paper, descending, n-BuOH–HOAc–H_2O, 4:1:2). The band was excised, extracted with water, and concentrated to a powder. The samples were then compared for their capacity to interfere with the formation of N-acetylphenylalanyl puromycin from N-acetylphenylalanyl RNA and puromycin in a ribosomal system derived from *E. coli*.[15a] Both samples inhibited this reaction by 58 ± 3% at 4×10^{-5} M.[15b] The growth inhibitory potency of synthetic Gougerotin against *E. coli* B was found to be the same as that exhibited by the natural antibiotic (50% inhibition at 4×10^{-5} M).[15c]

On the basis of the physicochemical and biological comparisons, it is concluded that the synthetic compound **7** is identical with Gougerotin. Since we have previously reported[8] the preparation of a C substance from D-galactose, the synthesis of Gougerotin presently described constitutes a total synthesis of this nucleoside antibiotic. The syntheses of analogs of Gougerotin and the determination of their biological activities are in progress and will be reported elsewhere.

Acknowledgment. This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. 08748).

(14) The natural Gougerotin sample was found to contain a faster migrating, fluorescent impurity which was removed by this process.

(15) (a) See C. Coutsogeorgopoulos, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **31**, 3603 (1972), for details of this system. (b) Personal communication from Dr. C. Coutsogeorgopoulos, Roswell Park Memorial Institute. (c) Personal communication from Dr. Alex Bloch, Roswell Park Memorial Institute, Buffalo, N. Y.

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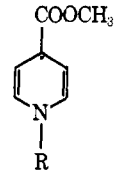
Associated Triplet States of Pyridinyl Radicals

Sir:

Pyridinyl radicals **1** associate intermolecularily to form diamagnetic dimer **2**, in which the self-association of the radicals has been interpreted in terms of charge-transfer complex formation.[1] Kosower and Waits[2] have found that a small amount of triplet dimer **3** is in equilibrium with the singlet dimer **2**. A triplet state has also been recognized for a two-spin system of 1,1′-ethylenebis(4-carbomethoxypyridinyl) diradical in 2-methyltetrahydrofuran (MTHF) glass.[3]



1a, R = CH_3
b, R = CH_2CH_3
2, Py·Py· singlet dimer
3, Py·Py· triplet dimer
(Py· = **1**)

We now report the observation of two triplet states in the self-association of pyridinyl radicals in MTHF glass. The results relate to the formation of associated triplet state from the singlet dimer by light irradiation and the complex formation of the radical with sodium iodide.

The epr spectrum of pure 4-carbomethoxy-1-methyl-(or ethyl)pyridinyl (**1a** or **1b**) in MTHF glass (α solution) measured in a dark room showed three pairs of shoulders on both sides of a strong $g = 2$ signal (at 3295 G) due to monoradicals. The spectrum is shown in Figure 1a. This is the indication of the existence of a triplet state which was previously suggested by Kosower and Waits.[2] Appearance of the $\Delta M = 2$ transition in the epr spectrum at 1645 G clearly proves the triplet. The intensities of the three pairs of lines decrease proportionally with decreasing concentration and disappear at around 3×10^{-3} mol/l. The zero-field parameters[4] estimated roughly are $D = 0.0098$ cm^{-1} and $E = 0.0011$ cm^{-1}, with $D + 3E = 141$ G and $2D = 202$ G. The values are similar for both radicals **1a** and **1b**. The D value is consistent with a spin–spin dipolar interaction for an average separation of 6.5 Å, using the relation $D = -(3/2)g^2\beta^2r^{-3}$.[5]

Irradiation of the α solution at 77°K with visible light added a new triplet signal (B_T signal) to the epr spectrum, as seen in Figure 1b. The intensity of the signal at 1645 G increased, while the signal intensity in the $g = 2$ region did not vary after the irradiation. The B_T signal lived for over 10 hr at 77°K after the light was shut off. Moreover, the phenomenon is completely reproducible for a cycle of warming, cooling, and irradiation.

The B_T signal is observed with rather better resolution and substantially higher intensity when the radical solution is saturated with sodium iodide. The epr spectrum of this solution (β solution = α solution + NaI) at 77°K does not have any shoulders due to the A_T form at around the $g = 2$ signal. Irradiation of the solution with light added a triplet spectrum consisting of two pairs of lines at the same position as those which appeared in Figure 1b. The spectra are shown in Figure 2. The zero-field parameters for the B_T signal are $D = 0.0175 \pm 0.0005$ cm^{-1} and $E \approx 0$, with $2D = 373 \pm 5$ G, an average of the similar values for both radicals **1a** and **1b**. It is suggested from the 0 E value that the association of radicals (B_T form) has more

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