

= 7.65 Hz; $\delta\text{H-8}' = 7.788$, $\delta\text{H-7}' = 6.675$, $\delta\text{H-6}' = 7.182$, and $\delta\text{H-5}' = 6.955$.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 CH₂ 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 $J_{\text{CH}_2-\text{H-1}}$. Adding a second oscillator tuned to CH₂-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 $J_{\text{epi}} > J_{\text{peri}}$ 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, C₆D₆). 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, $\approx 50\%$ D) were evident in the nmr (C₆D₆, 88°) of VII. By direct comparison of deuterio-trimethylthermorubin A (VII) with II the OCH₃D_{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 OCH₃D_{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 Gougerotin¹

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 acid⁷ (a derivative of the carbohydrate moiety of Gougerotin) and of 1-(4-amino-4-deoxy- β -D-glucopyranosyluronic acid)cytosine⁸ (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 substance⁸ 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, $[\alpha]_D^{27} -14^\circ$ (*c* 1.0,

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(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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(28) Where coupling constants could not be obtained directly via such multiple irradiation experiments²⁰ 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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+53° (*c* 0.8, H₂O) [lit.⁸ mp 210–215° dec; [α]_D²⁵ +45°]. A sample of Gougerotin kindly provided by Iwaski showed [α]_D²⁷ +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 chromatographed¹⁴ (Whatman No. 1 paper, descending, *n*-BuOH–HOAc–H₂O, 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 reported⁸ 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.

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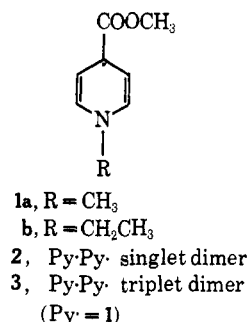
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Associated Triplet States of Pyridinyl Radicals

Sir:

Pyridinyl radicals 1 associate intermolecularly 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 Waits² 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

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 parameters⁴ 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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