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New Compounds: Organoboron Derivatives of Tetracyclines I: Synthesis of Carboxamido Derivative of Tetracycline with Perhydro-2-phenyl-1,3,6,2-dioxazaborocine

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Abstract □ An organoboron carboxamido derivative of tetracycline, designed for use in the $^{10}_{5}B$-thermal neutron-capture treatment of cancer, was synthesized under the conditions of the Mannich reaction using perhydro-2-phenyl-1,3,6,2-dioxazaborocine as the amine component. Spectral data (UV, IR, and NMR) for the compound and its hydrolytic stability are discussed.

Keyphrases □ Organoboron compounds—carboxamido derivative of tetracycline synthesized, UV, IR, and NMR spectral data and hydrolytic stability evaluated □ Tetracyclines—organoboron carboxamido derivative synthesized, UV, IR, and NMR spectral data and hydrolytic stability evaluated □ Antineoplastic agents, potential—organoboron carboxamido derivative of tetracycline synthesized, UV, IR, and NMR spectral data and hydrolytic stability evaluated

The avidity of the tetracycline antibiotics for various types of neoplastic cells (1–17) suggested their possible use as carrier molecules for selectively localizing the neutron-absorbing isotope of boron, $^{10}_{5}B$, in malignant tumors. The potential therapeutic value of boron thermal neutron capture *in situ* was first discussed by Locher (18) in 1936.

BACKGROUND

Successful use of a boron compound in the neutron-capture treatment of cancer requires its selective uptake by neoplastic cells and its retention for a sufficient period to allow for the clearance of the compound from other body tissues prior to thermal neutron exposure (19–21). Boron was recognized early as an ideal element for this purpose because of the large neutron-capture cross section of its naturally occurring $^{10}_{5}B$-isotope. The energy released from the neutron-capture reaction, $^{10}_{5}B + {}^{1}_{0}n \rightarrow [^{11}_{5}B] \rightarrow {}^{7}_{3}Li + {}^{4}_{2}He + 2.4$ Mev, where $^{1}_{0}n$ represents thermal or slow neutrons having energies of about 0.025 ev, is sufficient to cause localized destruction of boron-containing tumor cells.

Numerous reports on the preparation and evaluation of organoboron compounds for possible use in the neutron-capture treatment of cancer have appeared. With few exceptions, however, the results have been disappointing, primarily because the compounds lacked the necessary specificity to achieve acceptable ratios of tumor to normal tissue boron concentrations.

Because of the specific binding of tetracycline to ribosomes (22–28), tetracycline should be an ideal vehicle for transporting the fissionable element to rapidly proliferating neoplastic cells. Although the precise nature of the interaction between tetracycline and ribosomes remains to be established (24), suitable boronated derivatives might exhibit similar ribosomal binding characteristics. On this basis, the design and syntheses of boron-containing derivatives of the tetracyclines were undertaken. One borocine derivative[1] showed potential in neutron-capture experiments with adenocarcinoma-bearing mice (29), and the results of a human neoplastic cell culture study (30) suggested that the compound might be interacting at the ribosomal level.

The subject of this report is the synthesis of a new borocine Mannich-base derivative of tetracycline, characterized as 4-dimethylamino - 1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-*N*-[(perhydro-2-phenyl-1,3,6,2-dioxazaborocin -6-yl)methyl]-2-naphthacenecarboxamide (III). The Mannich base was prepared from tetracycline (I), perhydro-2-phenyl-1,3,6,2-dioxazaborocine (II), and paraformaldehyde, $(CH_2O)_n$, using a modification of the methods of Gottstein *et al.* (31) for preparing carboxamido derivatives of I (Scheme I).

EXPERIMENTAL[2]

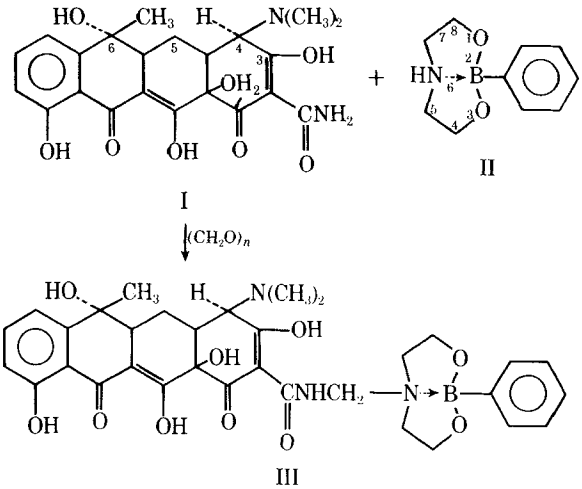
Perhydro-2-phenyl-1,3,6,2-dioxazaborocine (II)—This compound was prepared according to the method of Musgrave and Park (32) from 2,2′-iminodiethanol and phenylboronic acid, mp 214° [lit. mp 209.5–210° (32) and 214–215° (33)]; IR (KBr): ν_{max} 3100 (NH), 2900, 2850 (CH), 1200 (N → B) (34), 1175 (CO), 920 (N → B) (34), 750, and 705 (Ar) cm^{-1}; NMR (deuteromethanol): δ 2.55–3.17 (m, 4H), 3.47–4.10 (m, 4H), 4.77 (s, 1H), and 7.07–7.67 (m, 5H) ppm.

Compound III—A mixture of 4.32 g (8.68 mmoles) of I, 1.66 g (8.68 mmoles) of II, and 0.312 g (10.4 mmoles) of paraformaldehyde in 200 ml of absolute ethanol was gradually heated to reflux with stirring over 1 hr; nearly all solids dissolved during this time. The hot mixture was filtered, and the filtrate, after standing in the cold overnight, yielded 4.75 g (80%) of III as a water-soluble (~25%), yellow, amorphous solid, mp 300° dec.; $[\alpha]_D^{25}$ −156° (c 0.907, water); UV (methanol): λ 268 (log *a* 4.31) and 366 (4.26) nm.

Anal.—Calc. for $C_{33}H_{38}BN_3O_{10}\cdot 2H_2O$: C, 57.99; H, 6.19; B, 1.58; N, 6.16. Found: C, 58.09; H, 6.16; B, 1.49; N, 5.97. Equivalent weight by titration (perchloric acid in acetic acid) is 340, and water content (Karl Fischer) is 5.5% ($C_{33}H_{38}BN_3O_{10}\cdot 2H_2O$, mol. wt. 683.53, requires 342 and 5.3%, respectively).

[1] The chemical characterization of this compound will be the subject of another report.

[2] Melting points were determined on a Kofler micro hot stage and are uncorrected. Elemental analyses were performed by the Galbraith Laboratories, Knoxville, Tenn. UV and IR spectra were recorded on Hitachi P-E 139 and Beckman IR-5A and IR-10 spectrophotometers. NMR spectra were recorded on Varian A-60 and T-60 instruments with tetramethylsilane as the internal standard. Mass spectra were recorded on an A.E.I. MS9 instrument.



Scheme I

RESULTS AND DISCUSSION

The partial NMR spectra of I and III in deuteropyridine are shown in Fig. 1. The chemical shifts observed for the protons of the C-6 methyl and C-4 dimethylamino groups (δ 1.75 and 2.51) of I were in excellent agreement with previously published values (35). The broad singlet centered at δ 2.54 (6H) and assigned to the methyl protons of the C-4 dimethylamino group of III was partially overlaid by the downfield multiplet due to the C-5 and C-7 methylene protons of the borocine ring of II. The unobstructed broad singlet centered at δ 4.39 (2H) in the spectrum of III was assigned to the methylene protons of the NCH_2N group (calculated value, δ 4.25). This assignment is supported by NMR studies on model Mannich bases[3].

Overlapping of absorption bands in the IR spectrum of III virtually precluded definitive assignments of group frequencies. For example, the expected differences in absorption in the 1700–1500-cm^{-1} region due to the secondary amido group of III as compared to that of the primary amido group of I were obscured by extensive overlapping of bands (Fig. 2). However, with a sample of I in the reference beam, a difference

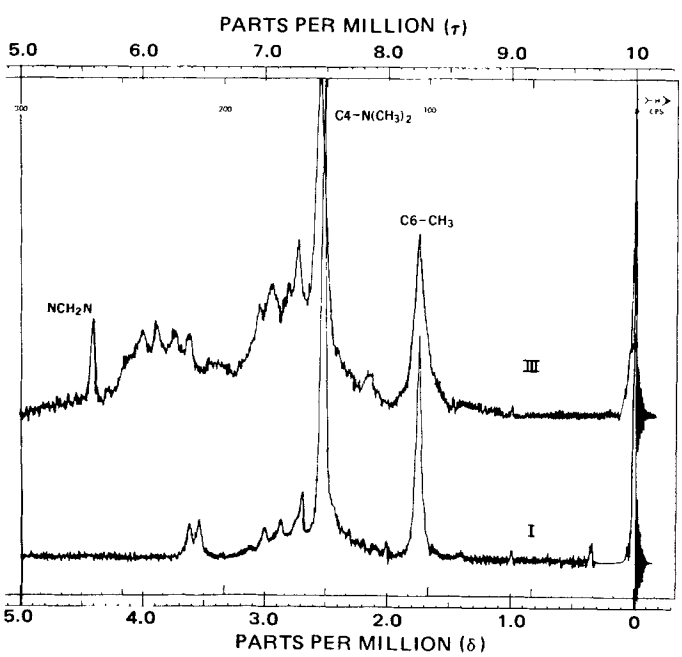


Figure 1—*Partial NMR spectra (deuteropyridine) of I (lower curve) and III (upper curve).*

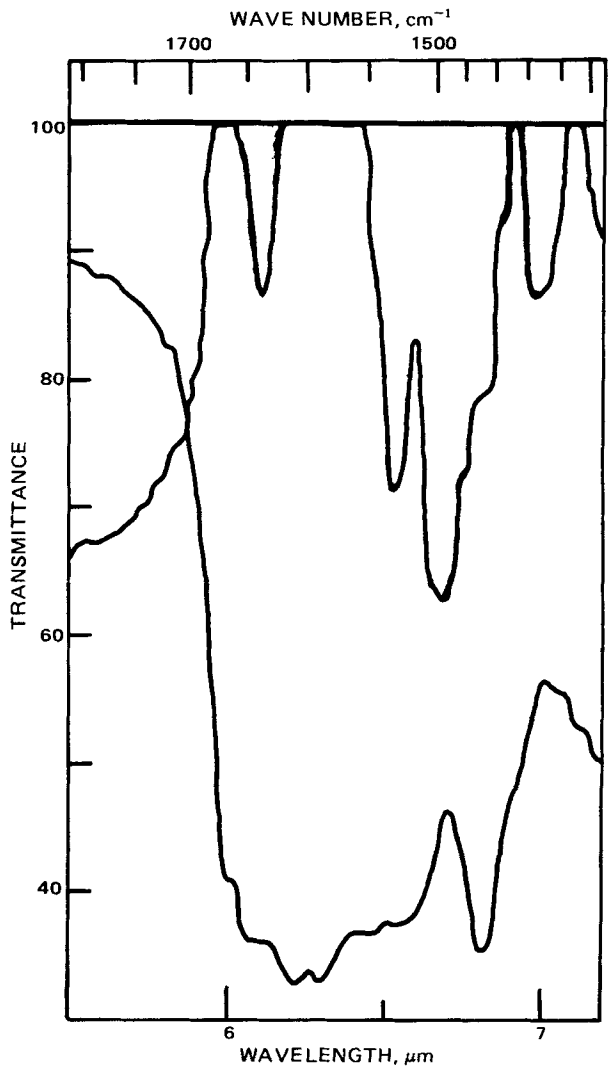


Figure 2—*Partial IR spectrum of III (lower curve) and its difference spectrum (upper curve).*

spectrum (Fig. 2) was obtained that clearly showed maxima at 1640 and 1530 cm^{-1}, to which the amide I and amide II bands, respectively, of the secondary amido group were assigned (36).

Except for slight differences in a_{max} values, the UV spectrum of III is identical to that of I. This finding has been taken as evidence that alkylation of the phenolic D ring of I did not occur as an alternative to alkylation of the amido group, since the former reaction could be expected to cause a detectable bathochromic shift of the aromatic B band.

Aqueous solutions of III changed in color from yellow to reddish-brown on standing at room temperature and unprotected from light for more than a few days. That these changes were preceded by hydrolysis was demonstrated clearly by changes in the NMR spectra of the solutions with time. In the presence of acid, the hydrolysis rate was accelerated greatly. For example, almost complete hydrolysis occurred when an aqueous solution of III (pH ~ 7) was adjusted to pH 2–3 with hydrochloric acid. This pH resulted in the precipitation of phenylboronic acid. Adjustment of the filtrate to pH 6 resulted in the precipitation of pure I. Further adjustment of the filtrate to pH 7 resulted in the precipitation of an additional small amount of I contaminated with III. The identity of recovered I was established by comparison of its melting point and IR, NMR, and mass spectra (70 ev), *m/e* 444 (M^+), with those of an authentic sample. Attempts to obtain a mass spectrum of III, using the direct insertion technique, were unsuccessful.

[3] Unpublished results.

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COMMUNICATIONS

Strain-Gauge Wheatstone Bridge Design for Automatic Capsule-Filling Machine

Keyphrases □ Capsule-filling machine, automatic—strain gauges, explanation of activity □ Instrumentation—automatic capsule-filling machine, explanation of activity of strain gauges

To the Editor:

A recent publication (1) described the application of strain gauges for determining compaction and ejection force in an automated capsule-filling machine. The redesign of the dosator piston for the installation of strain gauges resulted in the sensitive and linear measurement of force applied to the dosator piston. However, the design in which the strain gauges were applied does not provide a pair of passive gauge elements. The two gauge elements mounted perpendicular to the piston axis were described as temperature-compensating gauges and passive arms in the Wheatstone bridge circuit. In fact, these gauge elements in that design are active due to the phenomenon known as the Poisson effect (2). This effect is described in the following example.

When a material undergoes compression resulting in longitudinal compression strain, it also undergoes transverse tension strain (lateral strain). The relationship between the lateral and longitudinal strain is called the Poisson ratio (μ) and, for a given material, is relatively constant within the proportional limits for that material:

$$\mu = \frac{\text{lateral strain}}{\text{longitudinal strain}} \qquad \text{(Eq. 1)}$$

The expected sensitivity for this strain-gauge design can be calculated as follows. Young's modulus (E) for type 304 stainless steel, which is used in the instrumented dosator, is 1.97×10^6 kg cm^{-2} (3). Young's modulus is expressed mathematically as:

$$E = \frac{\sigma}{\epsilon} \qquad \text{(Eq. 2)}$$