C-N bond in the *tert*-butyl compound **2** relative to the Si-N bond in the disilyl analog **1** might, however, cause greater steric interaction with the $CF_3$ groups in the rotational transition state resulting in a higher barrier to N-P rotation.

The results of this study have a bearing on a report by Scherer and Schieder[8] in which the preparation of the phosphine imine, **3**, was described. Assignment of the imine

$$(t\text{-Bu})_2\text{PN(H)SiMe}_3 \xrightarrow[2.\ \text{Me}_3\text{SiCl}]{1.\ n\text{-BuLi}} (t\text{-Bu})_2\text{P}(\text{SiMe}_3)\text{=NSiMe}_3$$

**3**

structure to **3** was based largely on the observation of two different $Me_3Si$ signals, a doublet ($J_{PSiCH}$ = 2.2 Hz) and a singlet ($J_{PNSiCH}$ = 0.0), of equal area in the $^1H$ spectrum. In light of the high N-P rotational barriers and preferential phosphorus couplings reported here for the aminophosphines **1** and **2**, the spectrum of **3** would also be consistent with the isomeric aminophosphine structure $(t\text{-Bu})_2\text{PN}(\text{SiMe}_3)_2$ in which nonequivalent $Me_3Si$ groups result from hindered rotation about the N-P bond.

**Acknowledgment.** The authors are grateful to the National Science Foundation (Grant GP 38027X) and the Robert A. Welch Foundation for generous financial support.

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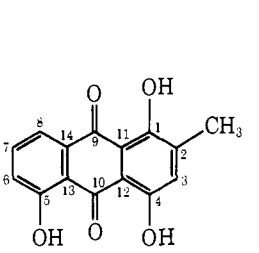
*Received May 16, 1975*

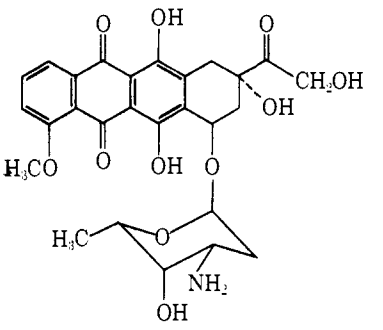
## A $^{13}C$ Nuclear Magnetic Resonance Study of the Biosynthesis of Islandicin from $^{13}CH_3{}^{13}CO_2Na$

*Sir:*

Islandicin (**1**), a red pigment isolated[1] from the mold *P. islandicum* may be viewed as a biosynthetic prototype of the potent anticancer antibiotic adriamycin (**2**).[2] The former has been shown by Gatenbeck[3] to be biosynthesized

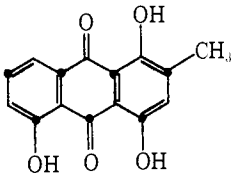


**1**

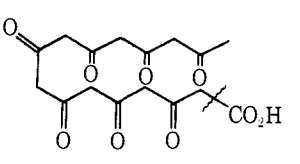


**2**

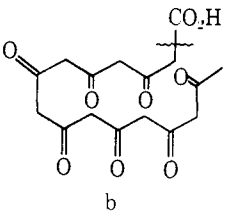
from a polyketide precursor. His experiments demonstrated that incorporation of $CH_3{}^{14}CO_2Na$ gives the following labeling pattern



which we have confirmed through incorporation of $CH_3{}^{13}CO_2Na$ and subsequent $^{13}C$ NMR. This pattern could arise from one of two possible foldings of a polyketide chain, a or b. We have demonstrated via the Tanabe tech-



a



b

nique,[4] the use of $^{13}C$ doubly labeled acetate ($^{13}CH_3{}^{13}CO_2Na$, 90% enriched), that islandicin is biosynthesized via configuration a.

*P. islandicum* Sopp was obtained from ATCC (no. 10127) and was maintained on Czapek-Dox-2% agar at 19-24°C. Petri dishes (100 × 15 mm) containing the growing organism were pulsed daily, each with 0.5 ml of $^{13}CH_3{}^{13}CO_2Na$ (8 mg/ml) from day 7 through day 16. Cultures were harvested[1] after 24 days, and the islandicin was purified by chromatography (benzene-silica gel) and sublimation. The isolated product (>90% pure) was chemically converted ($Ac_2O$/py) to the triacetate prior to $^{13}C$ NMR experiments. A small amount of $^{14}CH_3CO_2Na$ was added along with the $^{13}C$ acetate to accurately determine the incorporation level (2.0-2.5%).

Conversion of islandicin to the triacetate increased its solubility in $CDCl_3$ and made possible the use of $Cr(acac)_3$[5] for the $^{13}C$ NMR experiments. Under these conditions all the $^{13}C$ NMR signals were of approximately the same height (Figure 1A) which ensured that enrichment of carbon by labeled acetate would be readily apparent. This use of $Cr(acac)_3$ was especially helpful in determining incorporation levels and positions of singly-labeled precursors. The chemical shifts of islandicin triacetate are given in Table I and are based on known chemical shift data,[6] comparison with a number of model compounds (1,2-, 1,4-, 1,5-, 1,8-diacetoxyanthraquinones, 5-hydroxy-1,4-naphthaquinone and its corresponding acetate), and off-resonance decoupling experiments. C-1 was distinguished from C-4 and C-5 in off-resonance irradiation spectra where the former appeared as a sharp singlet and the two latter as broad signals; C-4 and C-5 were assigned by comparison of the spectrum

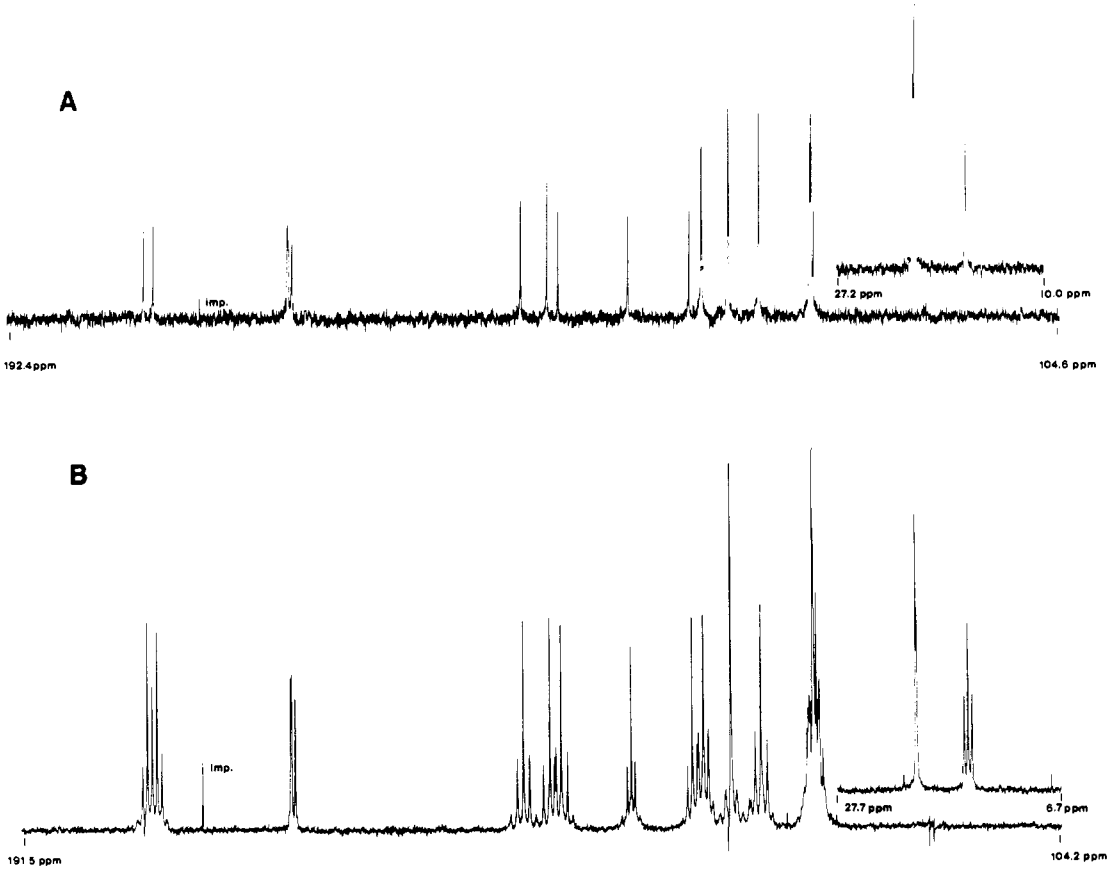



Figure 1. Proton noise-decoupled FT $^{13}C$ NMR spectrum of islandicin triacetate (20K transients; 3.0 sec pulse delay): (A) from $^{13}C$ natural abundance (73 mg + 15 mg of $Cr(acac)_3$), (B) from $^{13}CH_3{}^{13}CO_2Na$ enrichment (152 mg + 40 mg of $Cr(acac)_3$).

Table I. Assignment of Carbon Chemical Shifts in Islandicin Triacetate

| Carbon | Chemical shift (δ-internal TMS) | Carbon | Chemical shift (δ-internal TMS) |
|---|---|---|---|
| $C_2$-methyl | 16.6 | C-8 | 125.3 |
| C-1 | 146.4 | C-9 | 181.2 |
| C-2 | 140.6 | C-10 | 180.4 |
| C-3 | 132.2 | C-11 | 125.4 |
| C-4 | 147.4 | C-12 | 125.1 |
| C-5 | 149.6 | C-13 | 125.3 |
| C-6 | 129.7 | C-14 | 135.5 |
| C-7 | 134.5 | Acetate carbonyls | 169.1–168.8 |
| | | Acetate methyls | 21.0–20.9 |

with that of 1,4-diacetoxyanthraquinone. This, plus comparison with the spectrum of 1,8-diacetoxyanthraquinone and with that of 1,2-diacetoxyanthraquinone, led to assignment of C-9 and C-10 given in Table I. C-11 and C-12 were distinguished by experiments using singly labeled acetate; C-11 was derived from C-1 of acetate and C-12 from C-2 of acetate. Their accuracy was further confirmed by observation of a consistent coupling pattern in the spectrum of islandicin derived from doubly labeled acetate.

The FT $^{13}C$ NMR spectrum (Bruker WH 270) of enriched islandicin triacetate (Figure 1B) indicated a singlet for C-3 and a coupling pattern for the remaining carbons which confirmed the folding pattern a as involved in the biosynthetic pathway. $^{13}C$–$^{13}C$ coupling constants were determined for the following pairs of carbon atoms: $J_{CH_3\text{-}C\text{-}2} = 44.4$; $J_{4,12} = 71.2$; $J_{10,13} = 56.4$; $J_{5,6} = 70.2$; $J_{7,8} = 56.4$; $J_{14,9} = 54.6$; $J_{11,1} = 73.1$ Hz.

Close inspection of the enriched sample spectrum (Figure 1B) shows that most peaks, in particular C-3, have associated with the small symmetrically placed satellite pairs. These are of the expected intensity for multiply labeled species and apparently represent neither incursion of alternate biosynthetic pathways nor machine anomalies. We conclude this for the following reasons: (a) the C-1 resonance shows this effect and should not were configuration b operable, since the configuration would result in a singlet for this resonance; (b) the acetate carbonyl and methyl resonances do not show this effect (they probably would if the cause were machine anomalies); and (c) the C-2 methyl does not show this effect because it can have only one enriched neighboring carbon.

While it is thus unlikely that these satellite peaks arise from an alternative biosynthetic pathway, there are several ways in which multiply labeled species can arise. (1) The inherent probability of incorporation of labeled acetate at adjacent positions at this level of incorporation. The satellite peaks are calculated to be ca. 3–4% of the main peak at the observed level of incorporation. (2) The presence of a substantial preformed pool[7] of islandicin at the time of initiation of feeding. This would give rise to an enhanced intensity of the satellite peaks over that expected on the basis of average acetate incorporation. No perceptible islandicin is formed at time of initial pulsing, but any committed biosynthetic precursor (e.g., a polyketide) would give the same effect. (3) A lag period after initial pulsing during which exogenous acetate is not incorporated but islandicin is formed.

All of these are possible but in the absence of accurate intregration possibilities (2) and (3) above, which predict an enhanced intensity of the satellite peaks, can only be suggested.

These results demonstrate unequivocally that of the two pathways (a and b) only the former is correct. Numerous other possibilities are also ruled out.

**Acknowledgment.** This work was supported in part by the National Institutes of Health.

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## Interaction of *cis*-Diammineplatinum(II) with Nucleosides. Evidence for Bifunctional Electrophilic Attack and Base Stacking in the Binding to Inosine

*Sir:*

On the basis of Raman and $^1$H NMR studies on the reactions of *cis*-$(H_3N)_2Pt^{II}$ and $enPt^{II}$ with nucleosides in aqueous solution, we have obtained evidence for a strong bifunctional interaction with inosine in neutral solution which stabilizes the normal stacking of the bases. Coordination of the bifunctional platinum(II) electrophiles in neutral solution is considerably more complex than has been assumed in earlier work. Because of the proton loss from inosine and, by analogy, guanosine accompanied by stacking, it is questionable how much relevance studies on nucleosides and mononucleotides have to the stereochemistry of the interaction of bifunctional electrophiles with native polynucleotides.

Since the discovery by Rosenberg and coworkers[1] in 1969 that *cis*-$[PtCl_2(NH_3)_2]$ was a potent antitumor agent against Sarcoma 180 in Swiss White mice, there has been considerable interest in the activity of compounds of this type.[2-6] Studies of tissue cultures,[7-8] viral inhibition,[9] and bacterial transforming DNA[10] in the presence of active platinum(II) complexes suggest binding of the complex to nuclear DNA is responsible for the cytotoxic effect.

Reactions of *cis*-$[PtCl_2(NH_3)_2]$ and $[PtCl_2en]$ with nucleosides and nucleotides have been studied with uv,[11] NMR,[12,13] and mass spectra[14] and the reactions with dinucleotides by CD spectra.[15] With purines, coordination generally has been suggested to involve N(7) of inosine or guanosine and N(7) together with 6-$NH_2$ of adenosine; while for the pyrimidines, cytidine binds via N(3), and uridine or thymidine have been reported not to react. The primary reaction with native DNA's appears to be with guanine base.[16] Goodgame et al.[17] have reported the preliminary crystal structure of a nonstoichiometric complex $Na_{2.88}[Pt(NH_3)_2]_{0.56}(IMP)_2 \cdot 16H_2O$ which has two neutral inosines bound to platinum via N(7).

We have systematically mapped the perturbations of the base vibrations caused by heavy metal coordination at different sites.[18-22] These results, obtained with $CH_3Hg^{II}$ as a probe ion, can be used as an aid in interpreting Raman spectra for platinum binding.

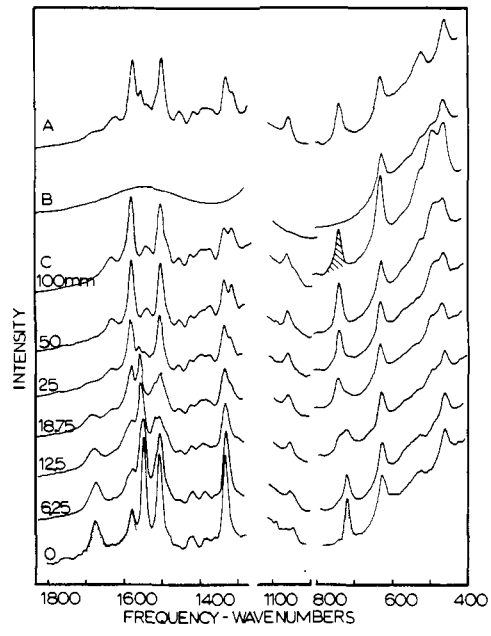


**Figure 1.** Raman spectra of 25 m*M* inosine, 25 m*M* $(H_3N)_2Pt^{II}$, and 25 m*M* inosine with $(H_3N)_2Pt^{II}$ or $enPt^{II}$ in $D_2O$ at 25°, pD 7.6: A, 25 m*M* Ino + 25 m*M* $enPt^{II}$; B, 25 m*M* $(H_3N)_2Pt^{II}$; C, Raman spectrophotometric titration of 25 m*M* inosine with $(H_3N)_2Pt^{II}$. Shaded bands indicate modes which can be used to determine the stoichiometry of the reactions.

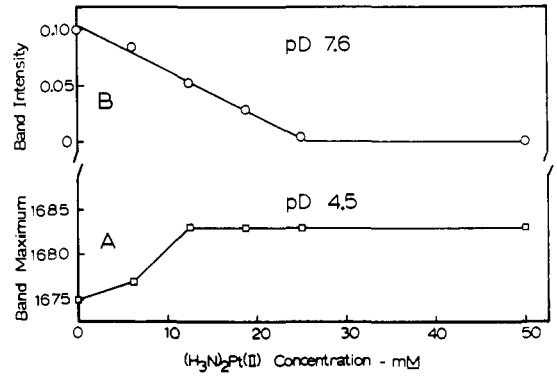


**Figure 2.** Changes in spectral parameters of 25 m*M* inosine upon the addition of $(H_3N)_2Pt^{II}$ in $D_2O$ at 25°. A (pD 4.5), values of the frequency at the maximum intensity showing the frequency shift in the 1650-1700-$cm^{-1}$ region. Overlapping bands are not resolved at 6.25 m*M* $(H_3N)_2Pt^{II}$. B (pD 7.6), values of the integrated intensity showing the disappearance of the 1675-$cm^{-1}$ band.

Raman spectra of solutions 50 m*M* in cytidine and in $(H_3N)_2Pt^{II}$, pH 7, 25°, 24 hr after mixing, are almost the same as those for cytidine quantitatively mercuriated at N(3).[18] Similar measurements with uridine show that there is only a slight decrease in the scattering at 1684 $cm^{-1}$ but none of the changes characteristic of quantitative mercuriation at N(3) with displacement of the proton. These results are consistent with previous reports on platinum coordination. We attribute the difference in platinum and methylmercury binding in these two cases to the reactions being kinetically controlled in the former, thermodynamically controlled in the latter case. In this respect platinum(II) binding resembles the reactions of alkylating agents.[23] Cytidine should be a good nucleophile using N(3); uridine is not, and the conjugate base of uridine ($pK_a$ = 9.5), a very good nucleophile, is not kinetically important in neutral solution.

Reaction of $(H_3N)_2Pt^{II}$ with inosine or guanosine at pH 7, 25°, by analogy, should involve electrophilic attack at N(7), although $CH_3Hg^{II}$ binds almost quantitatively both