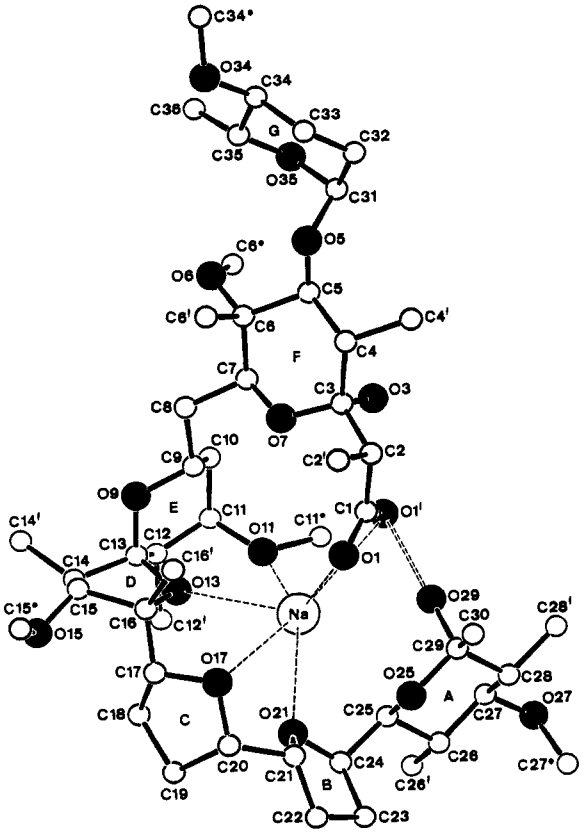

Figure 1.

The structure of the sodium salt of A204A is shown in Figure 1. The acetone solvate molecule, which is not shown, is weakly hydrogen bonded to the hydroxyl on ring F. The molecule has a central cavity of 5–6 Å in diameter formed by fastening the ends of the chain together with a hydrogen bond (double dotted line in Figure 1) between the hydroxyl on ring A and one of the carboxylate oxygen atoms. Six oxygen atoms of the antibiotic molecule lie less than 3.0 Å from the sodium ion and may be considered the principal ligands (single dotted lines). The structure of the complex is very similar to that found for grisorixin, the additional ring G in A204A being well removed from the region of the central cavity.

Acknowledgment. We wish to thank Mr. D. W. Smith for computer assistance.

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Reaction of Indoles with a Diazonium Salt (Fast Red B)

Sir:

Recent affinity labeling studies on rabbit antisaccharide antibodies[1] and staphylococcal nuclease[2] with diazotized aromatic amines have suggested—primarily on the evidence of visible absorption spectra—that a tryptophan residue has undergone reaction. This departure from the usual reaction of diazonium salts with protein-bound tyrosine, histidine, or lysine residues was first encountered in the alkaline coupling of proteins with diazotized arsanilic[3,4] or sulfanilic[5,6] acid. Reactions with indole,[5] indole-3-acetic acid,[4] and tryptophan[3,6] were also reported. Bernard[7] and Lillie[8] have discussed histochemical reactions of diazotized sulfanilic acid and *p*-nitroaniline, respectively, *vis-à-vis* tryptophan in proteins, reporting reactions with *N*-acetyltryptophan,[7] gramicidin,[7] and tryptamine.[8]

Fischer, in the first published study on the coupling of diazonium salts with indoles, observed[9] that 2-methylindole reacted rapidly to yield a crystalline 1:1 azo compound, whereas skatole, by presumably an alternate pathway, reacted more slowly to afford an uncharacterized product. This observation and Fischer's conclusion that reaction occurs preferentially at the indole 3 position were confirmed later[10] by Pauly and Gundermann with diazotized sulfanilic acid on indole, tryptophan, and 2-methyl-, 3-methyl- and 2,3-dimethylindole, and has been accepted in subsequent studies on the reaction of indole[11–17] and 2-methyl-[11,13–15] and 2-phenyl-[11] indole with various diazonium salts, though no additional experimental verification has been offered. A monosubstituted crystalline product from skatole and diazotized arsanilic acid has been described, solely by analogy to the pyrroles, as a coupling product at the 2 position.[15] In nearly all of these studies, the coupling reactions were conducted under weakly alkaline conditions, although there appears to be no justification for this practice.[18]

Because of our interest in the affinity labeling of proteins and certain histochemical applications we have examined the *acidic* and *neutral* reactions of skatole and 1,2- and 2,3-dimethylindole with a commercially available[19] diazonium salt (**1**) derived from 2-methoxy-4-nitroaniline.

The reaction with skatole was found to be pH dependent. When 2-deuterioskatole[20] was treated with 25%

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(2) P. Cuatrecacas, *J. Biol. Chem.*, **245**, 574 (1970).
(3) R. Kapeller-Adler and G. Boxer, *Biochem. Z.*, **285**, 55 (1936).
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(7) E. A. Bernard, *Gen. Cytochem. Methods*, **2**, 203 (1961).
(8) R. D. Lillie, "Histopathologic Technique and Practical Histochemistry," 3rd ed, McGraw-Hill, New York, N. Y., 1965, p 220.
(9) E. Fischer, *Ber.*, **19**, 2991 (1886).
(10) H. Pauly and K. Gundermann, *Ber.*, **41**, 3999 (1908).
(11) G. Plancher and E. Sonicini, *Gazz. Chim. Ital.*, **32**, 436 (1902).
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(15) Q. Mingoia, *Gazz. Chim. Ital.*, **60**, 134 (1930).
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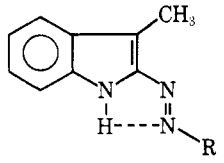
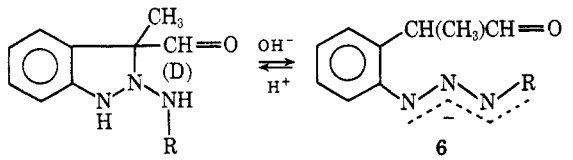
(18) Since simple indoles are un-ionized in the pH range under discussion, only the concentration of the reactive diazonium ion (RN_2^+) in equilibrium with unreactive $RN{=}NO^-$ is pH sensitive. As the pH increases the concentration of RN_2^+ decreases; *cf.* H. Zollinger, "Azo and Diazo Chemistry—Aliphatic and Aromatic Compounds," Interscience, New York, N. Y., 1961, p 47. Lillie has reported[8] a diazo coupling reaction with tryptamine at pH 4.
(19) Fast Red Salt B, Verona Dyestuffs, Union, N. J. 07083. Standardization either by comparison of the OD_{372} of a weighed amount in ethanol *vs.* a freshly prepared solution of diazotized (HCl) *p*-nitro-*o*-anisidine *or* coupling of a weighed amount with an excess of aniline and measurement of the OD_{530} in alkaline 50% aqueous dioxane gives 18–19% *p*-nitro-*o*-anisidine equivalents by weight.
(20) T. F. Spande, A. Fontana, and B. Witkop, unpublished work.

excess **1** in 50% aqueous dioxane, at pH 3.0, the major product (66% yield) **2** was a brick red (λ_{max}^{EtOH} 460 nm

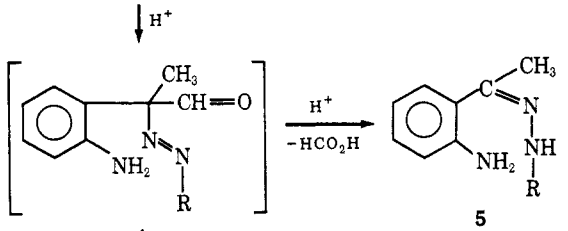


2

R = 2-methoxy-4-nitrophenyl



3 **6**

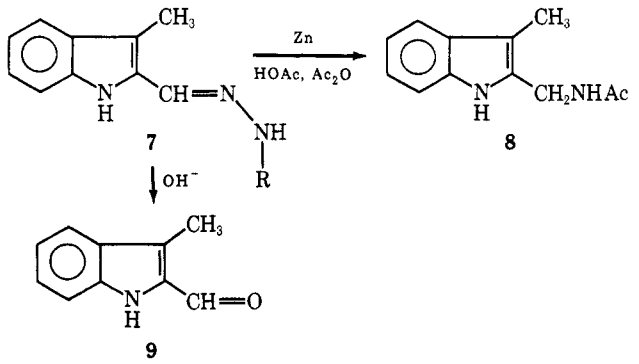


4 **5**

(log ϵ 4.44)), crystalline (mp 186–187°), 1:1 azo compound which had lost deuterium (*m/e* 310) while a minor, yellow (λ_{max}^{EtOH} 395 nm (log ϵ 4.39)) product **3**, double mp 192–195, 218–221°, evidently had retained deuterium as well as incorporated the elements of water (*m/e* 329). At pH 6–7, the proportions of **2** and **3** were reversed with **3** now predominating (42% yield) and only traces of **2**.

The 2-azo structure **2** for the red compound is supported by the labeling experiment, by pmr[21] (seven aromatic protons between δ 7.0 and 8.0, three-proton singlets at δ 4.10 and 2.74 for OCH_3 and CH_3, respectively, and a broad one-proton peak at δ 8.90 (N–H, H-bonded?), exchangeable with D_2O), and by ir data [3460 (indole NH), 1540, 1325 (asymmetric and symmetric NO_2, respectively), and 1345 cm^{-1}] as well as reduction[22] (Zn–HOAc–Ac_2O) to 1-acetyl-2-acetamidoskatole (47%) [mp 147–149.5° (*m/e* 230, 188, 146 (base), 145); ir (3460 br, 1700, 1690 cm^{-1})], whose uv spectrum [$\lambda\lambda_{max}^{EtOH}$ 242, 292, 301 nm, λ_{sh} 265 (log $\epsilon\epsilon$ 4.15, 3.86, 3.85, 4.05)] and its change with base [$\lambda\lambda_{max}$ 292, 300 nm (log $\epsilon\epsilon$ 4.07, 4.05)] are very similar to that reported[23] for 1-acetyl-2-acetamidoindole. The unusual "dihydrotriazene" structure **3** is proposed for the yellow azo product and is supported by its rearrangement with loss of deuterium to **5** under mildly acid conditions—an example of a facile Japp–Klingemann deformylation[24] of presumed intermediate **4**. The phenylhydrazone **5** (mp 141–142°) was synthesized (88%) from *o*-aminoacetophenone and 2-methoxy-4-nitrophenylhydrazine[25] and had identical tlc behavior, mass, and ir spectra. Also supporting structure **3** is the reversible transformation with alkali to the triazene anion **6** whose uv spectrum ($\lambda_{max}^{50\%\ dioxane}$ 530 nm (log ϵ 4.35)) resembles closely the alkaline spectrum from the triazene derived from aniline and **1** (λ_{max} 530 nm (log ϵ 4.47)); mass spectral (loss of CHO and NHR); pmr [one proton δ 8.5 (CH=O), nine aromatic protons between δ 8.25 and 7.10, three-proton singlets at δ 4.03 and 2.42 (OCH_3 and CH_3, respectively)]; ir data [3380 (NH), 3330 br (NH?), 1685 (CHO), and 1590 (aniline ν^{C-N}), and 1530 and 1335 cm^{-1} (NO_2)].

2,3-Dimethylindole afforded solely the phenylhydrazone **7** (mp 251–252°; λ_{max} 340, 445 nm (log $\epsilon\epsilon$ 4.11, 4.49)) on reaction with **1** at *either* pH 3 or 6–7, and yields of 83 and 48%, respectively, were obtained. Structure **7** is supported by pmr [(DMSO-d_6) *ca.* seven aromatic protons between δ 8.10 and 6.85, three-proton singlets at δ 4.0 and 2.40 (OCH_3 and 3-CH_3, respectively), one-proton singlet at δ 8.59 (CH=N), and broad, one-proton peaks (N–H, exchangeable with D_2O) at δ 10.62 and 11.10], mass spectral (*m/e* 324, 177, 159, 157), and ir (3470 (indole NH), 3330 (NH), 1595 cm^{-1} (CH=N?)) data. Zinc dust reduction of **7** gave an oily material (50%) whose properties (*m/e* 202, 159, 143, 130; uv λ_{max}^{EtOH} 278, 282 (sh), 292 (sh) nm; ir 3450, 3410, 3300, 1660, 1520, 1455 cm^{-1}) fit 2-acetamidomethylskatole **8**. Alkaline hydrolysis of **7** affords 2-formylskatole **9**: mp 140–141° (lit. 136–139°,[26] 139–140°[27]); $\lambda\lambda_{max}$ 240, 316 (log $\epsilon\epsilon$ 4.33, 4.53); *m/e* 159, 160 (P, P + 1); ir 3450, 1645 cm^{-1}. **7** most



7 **8** **9**

probably arises by a 1,3 rearrangement[28] of an initially formed 3-arylazoindolenine (**10**) to the 2-arylazomethylindole (**11**) followed by isomerization; see ref 28 for three recent examples of this type of rearrangement.[29]

1,2-Dimethylindole with **1** produces (70%) a 3-azo derivative [mp 198–206°; $\lambda\lambda_{max}^{DMSO}$ 287, 467 nm (log $\epsilon\epsilon$ 4.00, 4.38); *m/e* 324] which exhibits *no* bathochromic

(21) In $CDCl_3$ unless indicated otherwise. Chemical shifts are in δ (parts per million) relative to tetramethylsilane as an internal standard.

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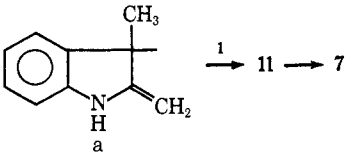
(29) A referee has suggested that **7** might arise by direct attack of **1** on **2** (in equilibrium with 2,3-dimethylindole). We have no evidence to conclusively rule this out but prefer the mechanism above since it is consistent with the known reactivity of the indole 3 position toward electrophiles and accounts for the formation of **7** under both acidic and neutral conditions. As suggested, the alternative mechanism would require proton exchange in the 2-methyl group of recovered starting material. Although this point has not been checked, we doubt whether such exchange would occur at pH 6–7.



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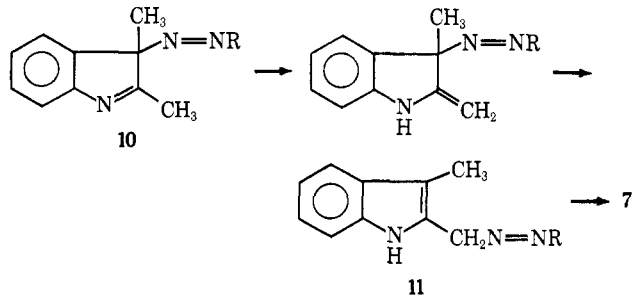

10

11

shift with alkali, confirming speculation in the literature that such shifts observed with 3-azo products from indole[16] or 2-methylindole[14] arise from removal of the indole N–H proton.

(30) (a) Laboratory of Chemistry; (b) Laboratory of Experimental Pathology.

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A New Boron Hydride, Pentadecaborane(23)

Sir:

A new crystalline boron hydride has been prepared in high yield by reaction of *i*-B_9H_{15} with B_6H_{10}. The air sensitive compound has moderate thermal stability.

In a typical reaction KB_9H_{14} (2.06 mmol) was treated with excess HCl at −78° for 30 min. The unreacted HCl was then distilled from the reactor by pumping for 3 hr at −78° yielding a mixture of solid *i*-B_9H_{15} and KCl as described elsewhere.[1] Hexaborane(10) (10.20 mmol) was condensed into the reactor and melted onto the frozen *i*-B_9H_{15} mixture, being certain to cover each portion of the solid with liquid B_6H_{10}. The mixture was warmed to 0° for 20 min. Hydrogen (2.00 mmol) and B_6H_{10} (8.12 mmol) were removed at −196 and 0°, respectively. The solid remaining in the reactor was extracted with CH_2Cl_2 and separated from KCl by filtration. Evaporation of the clear colorless solution gave crystalline $B_{15}H_{23}$ (2.01 mmol). The KCl was dissolved in water and precipitated with $AgNO_3$ yielding AgCl (1.86 mmol). Therefore, the reaction proceeded according to the following stoichiometry assuming quantitative yields of *i*-B_9H_{15}.

$$(1.00)\ i\text{-}B_9H_{15} + (1.01)\ B_6H_{10} = (0.97)\ H_2 + (0.98)\ B_{15}H_{23}$$

Hydrolysis of the borane with dilute HCl and titration of the boric acid as the D-mannitol complex resulted in hydrolytic H_2 to $B(OH)_3$ ratios of 2.265 and 2.265, calcd for $B_{15}H_{23}$, 2.267. Six determinations of the molecular weight by vapor pressure depression in CH_2Cl_2 solvent at 0 and +5° gave an average molecular weight of 204, calcd for $B_{15}H_{23}$, 185. The value is probably high due to slow evolution of hydrogen from the sample during the determinations.

The 70.6-MHz ^{11}B nmr spectrum measured in $CDCl_3$ is shown in Figure 1. The sets of peaks have integrated intensities of 1 (a):3 (b + c):3 (d + e):2 (f):2 (g + 2h):2 (i + j) in agreement with a borane molecule containing 15 boron atoms. Resolution of peaks d and e was accomplished by "artificial line narrowing" as described elsewhere.[2]

In view of the established basicity of B_6H_{10}[3,4] and the preparation of B_9H_{13}·ligand compounds from *i*-B_9H_{15},[1,5] we picture the hydride as an acid–base adduct, possibly one in which the two boron frameworks are held together by a three-center bond.

An X-ray crystallographic study, isotopic labeling, and other nmr studies, as well as a study of the chemistry of this hydride, are in progress. Furthermore, attempts to extend the reaction of B_6H_{10} to other boron hydride Lewis acids have already given encouraging results.

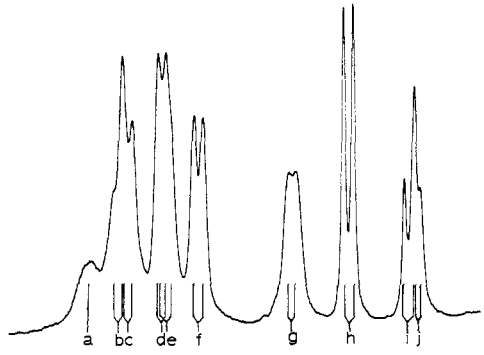

Figure 1. The 70.6-MHz ^{11}B nmr spectrum of $B_{15}H_{23}$ measured in $CDCl_3$. Chemical shifts (ppm referenced to $BF_3 \cdot Et_2O$) and coupling constants (+10 Hz) are as follows: a (−20.9), b (−14.3, 140), c (−12.0, 146), d (−4.5, 128), e (−3.1, 160), f (+3.6, 139), g (+24.4, 113), h (+36.9, 153), i (+50.5, 155), j (+52.4, 101).

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Resonance Raman Spectra of Vitamin B_{12} Derivatives[1]

Sir:

Resonance Raman spectroscopy offers promise as a sensitive structural probe for biological chromophores.[2] Excitation within an electronic absorption band can produce large enhancements of certain of the Raman bands of the absorbing molecule.[3] We have obtained Raman spectra of several vitamin B_{12} derivatives (Figure 1), in dilute solution (10^{-3}–10^{-4} *M*) using laser excitation (4880 Å) within the visible absorption bands of the molecules (Figure 2). The technique may be useful

(1) This investigation was supported by Public Health Service Grants GM-13498 and HL-12526.

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(3) J. Behringer in "Raman Spectroscopy," H. A. Syzmanski, Ed., Vol. I, Plenum Press, New York, N. Y., 1967, Chapter 6.