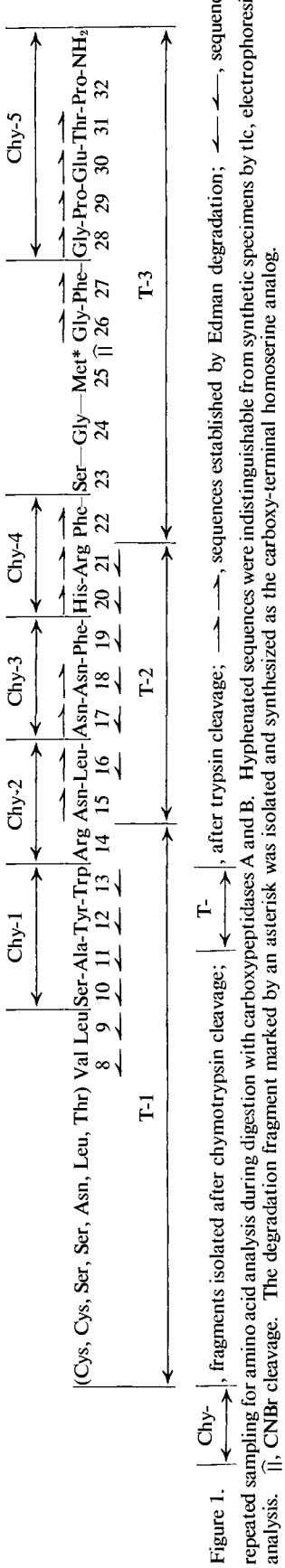

Figure 1. Chy-, fragments isolated after chymotrypsin cleavage; T-, after trypsin cleavage; ⟶, sequences established by Edman degradation; ⟵, sequences determined by repeated sampling for amino acid analysis during digestion with carboxypeptidases A and B. Hyphenated sequences were indistinguishable from synthetic specimens by tlc, electrophoresis, and amino acid analysis. Met*, CNBr cleavage. The degradation fragment marked by an asterisk was isolated and synthesized as the carboxy-terminal homoserine analog.

Peptide fragments isolated after cleavage with chymotrypsin are also shown in Figure 1. The important tripeptide 20–22 (Chy-4), which overlaps two tryptic fragments, was identified by Edman degradation and by synthesis. The tyrosyl–tryptophan bond in the chymotryptic fragment Chy-1 was stable to chymotrypsin both in the natural and in the synthetic tetrapeptide.

Treatment of the tryptic fragment 15–21 with thermolysin[9] afforded phenylalanylhistidylarginine as well as fragment 16–18. Degradation of fragment 22–32, obtained from trypsin cleavage, with CNBr gave phenylalanylserylglycylhomoserine as well as fragment 26–32. The presence of an amino-terminal phenylalanine in the tetrapeptide was shown by the dansylation procedure for end-group analysis.[10] It was confirmed by degradation with leucine aminopeptidase which also served to demonstrate the presence of serine in position 23. Moreover, the tetrapeptide was found to be indistinguishable from a synthetic specimen. Sequence 26–31 was established by Edman degradation of fragment 26–32. Leucine aminopeptidase confirmed the presence of the terminal sequence Gly-Phe (26–27).

Hydrolysis of thyrocalcitonin with 0.03 *N* HCl at 100° for 9 hr liberated leucine and aspartic acid as the only detectable amino acids. This result is consistent[11] with the presence of an Asn-Leu-Asn fragment in the hormone. The tryptic fragment 15–21 (T-2) was also found to be identical with a synthetic[7] specimen.

(9) H. Matsubara, R. M. Sasaki, and R. K. Chain, *Proc. Natl. Acad. Sci. U. S.*, **57**, 439 (1967).
(10) W. R. Gray and B. S. Hartley, *Biochem. J.*, **89**, 59P (1963).
(11) C. M. Tsung and H. Fraenkel-Conrat, *Biochemistry*, **4**, 793 (1965).

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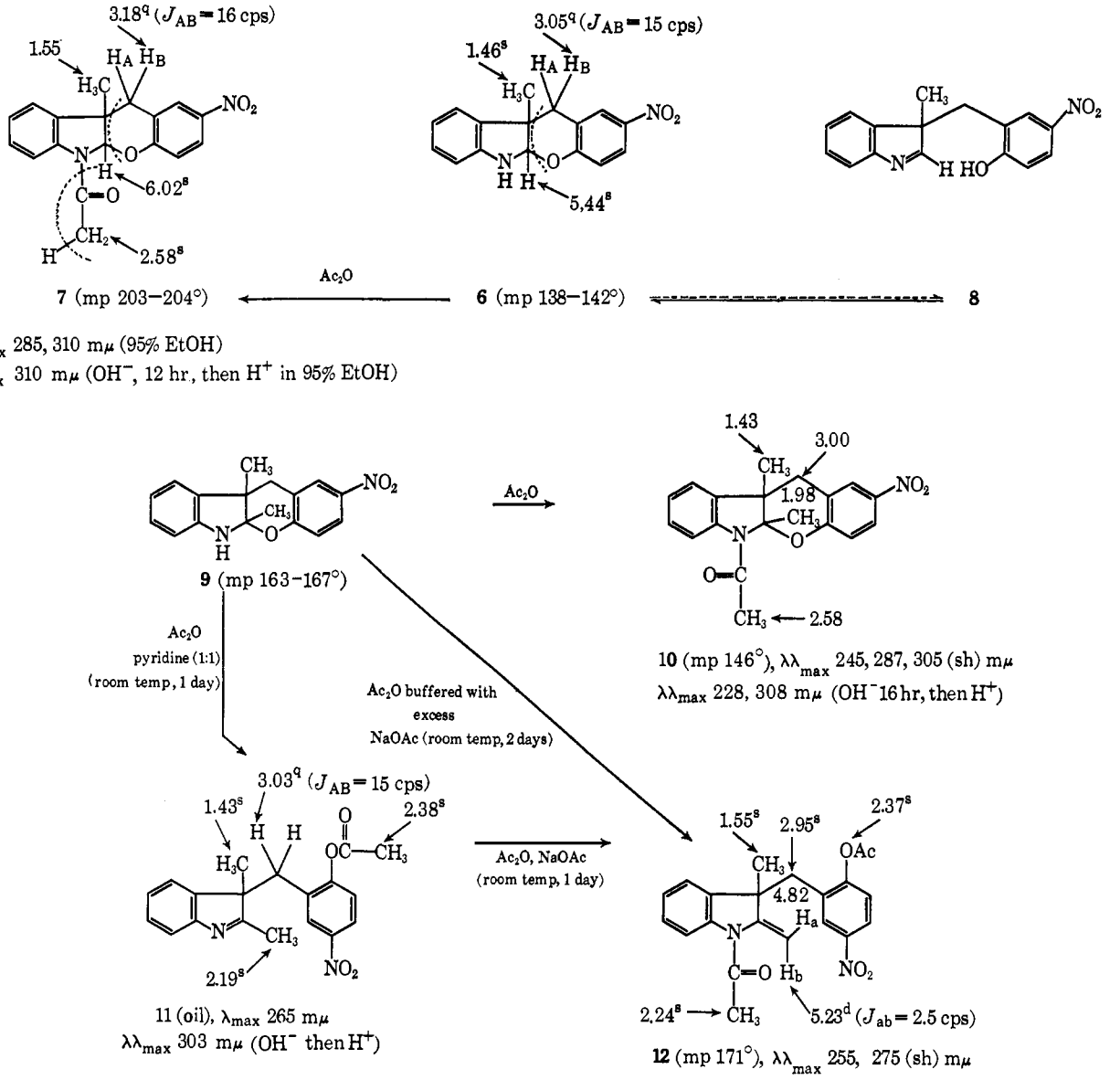
The Reaction of Derivatives of Tryptophan, Tryptamine, and Other Indoles with 2-Hydroxy-5-nitrobenzyl Bromide (Koshland's Reagent)

Sir:

Although interest in the highly selective reagent 2-hydroxy-5-nitrobenzyl bromide (**1**) for the rapid modification and assay of tryptophan in proteins continues,[1–7] there is no information on the structure of the reaction products.[7a] A recent report[8] on the complex reactions of the reagent with tryptophan, both free and bound in proteins, prompts us to communicate our findings on the reaction of **1** with skatole (**2**), 2,3-dimethylindole (**3**), N-acetyltryptamine (**4**), and N-acetyl-L-tryptophan methyl ester (**5**).

(1) D. E. Koshland, Jr., Y. D. Karkhanis, and H. G. Latham, *J. Am. Chem. Soc.*, **86**, 1445 (1964).
(2) H. R. Horton and D. E. Koshland, Jr., *ibid.*, **87**, 1126 (1965).
(3) T. A. Bewley and C. H. Li, *Nature*, **206**, 624 (1965).
(4) K. Yamagami and K. Schmid, *J. Biol. Chem.*, **242**, 4176 (1967).
(5) N. B. Oza and C. J. Martin, *Biochem. Biophys. Res. Commun.*, **26**, 7 (1967).
(6) M. Wilchek and B. Witkop, *ibid.*, **26**, 296 (1967).
(7) R. F. Steiner, *Arch. Biochem. Biophys.*, **115**, 257 (1966).
(7a) NOTE ADDED IN PROOF. Under somewhat different conditions 2-alkylation of skatole has been reported: M. Wakselman, G. Decodts, and M. Vilkas, *Compt. Rend.*, **266**, 1090 (1968).
(8) T. E. Barman and D. E. Koshland, Jr., *J. Biol. Chem.*, **242**, 5771 (1967).



When a solution of **1** in dry acetone was added to an equimolar quantity of **2** in 50% acetone–water (final composition 72% acetone), a homogeneous, yellow, crystalline material (mp 138–142° after recrystallization from 80% acetone) separated in 70% yield. Combustion analysis and mass spectrometry (*m/e* 282, parent molecular ion) established formula $C_{16}H_{14}N_2O_3$, *i.e.*, a 1:1 reaction product. Principal peaks were observed at *m/e* 132 and 131 (intensity ratio ≅ 3:1), equivalent to the loss from the parent ion of $C_7H_5NO_3$ and $C_7H_6NO_3$, respectively. The nmr spectrum ($CDCl_3$; TMS internal standard) supports structure **6**: a three-proton singlet at 1.46 ppm, a two-proton quartet (J_{AB} = 15 cps) at 3.05 ppm, a one-proton singlet at 5.44 ppm, a five-proton multiplet at 6.4–7.0 ppm, and a two-proton multiplet at 7.6–7.9 ppm. D_2O was without effect on this spectrum. The ir spectrum ($CHCl_3$) exhibited three principal peaks above 1525 cm^{-1} at 3450 (sharp), 1615, and 1585 cm^{-1}, the latter two being the most intense. On acetylation (Ac_2O, room temperature), a colorless microcrystalline N-acetate, **7** (mp 203–204°), was formed in quantitative yield. The analysis, parent molecular ion (*m/e* 324), and the ir (1670 cm^{-1}; 3450 cm^{-1} had disappeared) and nmr spectra [1.55 (3 H), singlet; 2.58 (3 H), singlet; 3.18 (2 H), quartet (J_{AB} = 16 cps); 6.02 (1 H), singlet; 6.8–7.3 (4 H), multiplet; 7.8–8.1 ppm (3 H), multiplet] support structure **7**. The dotted lines show the principal mode of cleavage in the mass spectrometer.

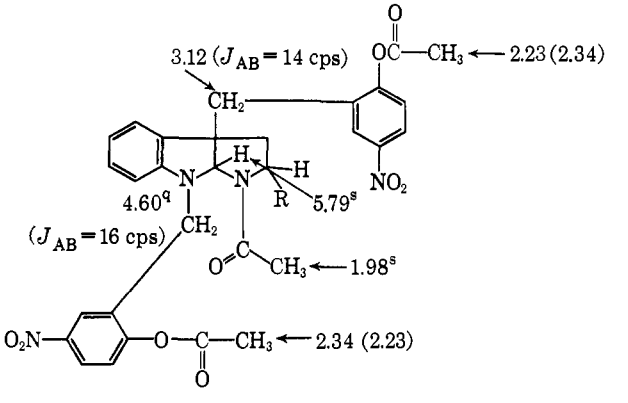
The upfield shift of the 3-methyl singlet from 2.30 ppm in **2** to 1.46 and 1.55 ppm in **6** and **7** provides compelling evidence for alkylation in the 3 position. Similarly, allyl bromide alkylates 1,2-dimethylindole to a mixture of two bis(alkylated) products, the major component being a 3,3-diallyl derivative.[9] The presence of a one-proton singlet at 5.44 (**6**) and 6.02 ppm (**7**) supports the carbinolamine structure[10] and rules out the isomeric indolenine **8**, which may well be the initial product of alkylation. Spontaneous cyclization of **8** should lead to the preferred *cis* ring juncture indicated for **6** and **7**.

2,3-Dimethylindole (**3**) under the same reaction conditions as **2** formed an initial adduct (**9**) with 1 mole of reagent. Acetylation of this product gave the three products, **10**, **11**, or **12**, depending upon the experimental conditions (see diagram). In each case, the

(9) K. R. Freter, *Can. J. Chem.*, **45**, 2628 (1967).
(10) D. Robinson, *J. Chem. Soc.*, **1503** (1964).

product was nearly homogeneous; **10** and **12** were crystallized directly. **11** was an unstable oily material which resisted crystallization but was smoothly converted to **12** by further acetylation with Ac_2O–NaOAc. Pertinent nmr assignments are indicated on the structures.

Preliminary experiments on the alkylation of N-acetyltryptamine (**4**) with 1 equiv of **1** indicated that the major product contained 2 moles of reagent. Accordingly the alkylation was carried out with 2 equiv of **1**. Purification was effected inadvertently during an acetylation attempt with Ac_2O (room temperature) when the major product crystallized directly from the acetylation mixture as a 1:1 complex with Ac_2O (60% yield). This material dissolved readily in $CHCl_3$ at room temperature and, after 20–30 min, deposited an almost insoluble 1:1 chloroform complex of the composition $C_{26}H_{24}N_4O_7$–$CHCl_3$. An ir spectrum (KBr) indicated associated phenolic stretching vibrations (3000–3400 cm^{-1}) and a hydrogen-bonded (tertiary?) amide carbonyl (1620 cm^{-1}). This material was acetylated with Ac_2O–pyridine (1:1) to give a quantitative yield of the N,O,O-triacetate **13**, $C_{30}H_{28}N_4O_9$, obtained solvent free on crystallization from EtOAc–hexane or as a 1:1 $CHCl_3$ complex from $CHCl_3$–hexane. A high-resolution mass measurement of the parent molecular ion indicated *m/e* 588.178 (calcd 588.183). A low-resolution mass spectrum was consistent with the consecutive loss of two units of *m/e* 194 each from the parent. The molecular extinction of 35,800 at 400 mμ in alkaline ethanol is consistent with a molecular weight of 707 for the chloroform complex of **13**, from which base forms 2 moles of the characteristic chromophore of the *p*-nitrophenoxide ion (ϵ_{410} 18,000). Structure **13**, though awaiting confirmation by X-ray crystallography, is preferred to other alternatives such as the open indolenine tautomer with N_b carrying acyl and benzyl groups.



13, R = H (mp 164–167°; $CHCl_3$ adduct, mp 96–100°)

14, R = COOMe (mp 95–105°)

$\lambda\lambda_{max}$ 252, 270 (sh) mμ

$\lambda\lambda_{max}$ (OH^- then H^+) 246, 315 mμ

Three products resulted when N-acetyl-L-tryptophan methyl ester (**5**) was treated with 2 equiv of the reagent and then acetylated with Ac_2O–pyridine (1:1 room temperature, 3 days). The major component (37% after silica chromatography) had the composition $C_{32}H_{30}N_4O_{11}$ (mp 95–105° after crystallization from ether–ligroin). The near identity of its uv spectrum with **13**, before and after base treatment, and many close similarities in its nmr spectrum (**13**) suggest the analogous structure **14**. Such a dihydroindole system would no longer show the reactivity that bound tryptophan shows toward N-bromosuccinimide.[6]

Acknowledgment. We wish to acknowledge our indebtedness to Dr. G. W. A. Milne for determining the exact mass for compound **13** and to Dr. Takashi Tokuyama for obtaining several of the nmr spectra.

(11) Fellow in the Visiting Program of the U. S. Public Health Service, 1967–1968.

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The Selective Photolysis of Dihydrothymidine

Sir:

Of all the building stones of DNA, thymine most easily undergoes photochemical transformations, such as dimerization[1-3] or possibly reduction,[4] events which are of genetic and mutagenic interest. This report shows that dihydrothymidine, in contrast to dihydrouridine, undergoes a selective "photochemical hydrolysis" of the Ciamician–Silber type[5] which, though well known for homocyclic systems,[6] is here demonstrated for the first time for a heterocyclic representative of biological significance.

In a typical run, a 2×10^{-2} *M* aqueous unbuffered solution of dihydropyrimidine (Ia or Ib) was irradiated with a 250-W high-pressure mercury lamp (Hanovia S654-36, no filter) which was surrounded by a cylindrical water-cooled quartz jacket. Two semicircular quartz vessels which surrounded the cooler and contained the sample were 3 cm from the light source. Irradiation of 1-dihydrothymidine (Ib)[7] for 30 hr led to complete disappearance of the uv absorption at 230 mμ. The photoproduct, a yellow oil, was lyophilized, chromatographed over silica gel, and eluted with chloroform–methanol (9:1). The three major fractions yielded (*S*)-(−)-dihydrothymine, mp 263° (Ia),[7] *n*-propylurea (VIa, 6%), mp 109° (*p*-nitrobenzoate mp 170–171° dec), and N_1-deoxyribosyl-N_1-*n*-propylurea (VIb, 64%), which on acid hydrolysis gave *n*-propylurea, mp 109°, and deoxyribose (diphenylamine test). Dihydrothymine on photolysis gave 75% *n*-propylurea (VIa) and 5% urea. *Under identical conditions the photolysis of dihydrouridine led only to minor cleavage of the ribosyl residue and to the isolation of 5% of dihydrouracil.*

There are two likely reaction mechanisms. Pathway A would begin with homolytic cleavage between the carbonyl group (position 4) and the ureido nitrogen

(1) R. O. Rahn, R. G. Shulman, and J. W. Longworth, *J. Chem. Phys.*, **45**, 2955 (1966).
(2) A. Wacker, *Progr. Nucleic Acid Res.*, **1**, 369 (1963).
(3) R. B. Setlow and W. L. Carrier, *J. Mol. Biol.*, **17**, 237 (1966).
(4) T. Yamane, B. J. Wyluda, and R. G. Shulman, *Proc. Natl. Acad. Sci. U. S.*, **58**, 439 (1967).
(5) G. Ciamician and P. Silber, *Ber.*, **43**, 1340 (1910).
(6) *Cf.* G. Quinkert, *Angew. Chem.*, 77, 229 (1965).
(7) Y. Kondo and B. Witkop, *J. Am. Chem. Soc.*, **90**, 764 (1968). The chemical evidence for the *S* configuration of the asymmetric center at C-4 of 1-dihydrothymidine has been supplemented and confirmed by a complete X-ray structure analysis (I. L. Karle, Naval Research Laboratory). There is evidence that *exposure to radiation by X-ray leads to partial conversion of dihydrothymidine to thymidine*, and probably of dihydrothymine [*cf.* S. Furberg and L. H. Jensen, *ibid.*, **90**, 470 (1968)] to thymine.