it was necessary to reindex data in the $P2_1/c$ system with corresponding lattice constants of $a = 9.310$, $b = 9.592$, $c = 11.750$, and $\beta = 140.09°$. All subsequent work was carried out in the new system.

The initial R value was 0.30 for the structure selected from the three-dimensional plot of the E map. Refinement using isotropic temperature factors led to a final value of 0.16. Introduction of anisotropic temperature factors on the chlorine atoms alone reduced the R factor to 0.10. The quality of data obtained with this crystal did not warrent further refinement.

Several new crystals were examined in an effort to obtain better data. Both crystals mounted directly in air and those mounted in sealed glass capillaries changed during the data collection process. In the case of one crystal mounted in a capillary, we were able to collect the complete set of redundant data on indices $\pm h$, k, and $\pm l$ using Mo radiation to a 2θ value of 30°. Three standard reflections were monitored once every 30 observations. All three standards decayed uniformly with time and at the end of data collection intensities of the standards had been reduced by 25%. At this time it was deemed that further data collection with this crystal was undesirable and no additional suitable crystals were available for study. All 981 observed reflections were used to calculate F^2 values. Data on the standard reflections were fitted to a linear decay curve by a least-squares procedure and the F^2 value of each reflection was corrected for the decay in crystal quality. Average values for a set of nonredundant data were calculated and in no case did independent observations differ by more than 2σ. The order of data collection was such that symmetry-equivalent reflections were symmetrically placed about the median data point and thus the excellent average values obtained suggested that the data were adequate for further refinement. The new set of 210 observations was used for isotropic refinement using the parameters determined for the previous data set, leading to a value of $R = 0.13$ when isotropic temperature factors were used for all atoms. Introduction of nonisotropic temperature factors for the chlorines alone led to an R value of 0.074 in the first cycle and ultimately to 0.071. Parameters for each atom at this stage of refinement are listed in Table I. Temperature factors are normal

Table I. Atomic Parameters Based in $P2_1/c$ Cell

Atom	X	Y	Z
Cl-1	0.7411	0.0010	0.4878
Cl-2	0.7152	0.8701	0.7607
N	0.6514	0.2834	0.0926
C-1	0.8753	0.1723	0.7882
C-2	0.2933	0.6791	0.4691
C-3	0.5138	0.7925	0.9304
B	0.5367	0.4394	0.9720

although slightly high for carbons (up to 8.2), as expected in slight compensation for missing hydrogen atoms Bond distances derived from these parameters are: BB, 1.72; BN, 1.75; BCl-1, 1.86; BCl-2, 1.89; NC-1, 1.57; NC-2, 1.54; NC-3, 1.53 Å. All angles are satisfactory at this stage of refinement.

(3) Q. Johnson, G. S. Smith, and E. Kahara, *Science*, **164**, 1163 1969).

At this moment we have no explanation for the molecular weight measurements made 20 years ago. We suggest that they were in error and point out that the chemically reactive nature of the molecule may have made valid measurements difficult. In light of both the present structural analysis and the new data on the mass spectrum of the total sample, we believe that the problem is completely resolved.[4,5]

(4) The authors regret the length of this communication produced by inclusion of details of experimental procedure and results that we do not believe to be of general interest. They are included only at the insistence of the referees and the editor. The reader is warned that the values listed in Table I may be slightly incorrect owing to both the incomplete data set used and the failure to find the nine hydrogens in the asymmetric unit. The basic structural conclusion is in no way affected by these technical limitations.

(5) This work was supported in part by the Atomic Energy Commission and in part by the National Science Foundation.

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Synthesis of Slaframine

Sir:

Slaframine, a metabolite isolated from the fungus *Rhizoctonia leguminicola*,[1] is responsible for producing salivation in cattle.[2] Studies on slaframine have indicated that it has potential value—both as a research tool, for the isolation of the acetylcholine receptor site, and as a medicinal agent for the treatment of the cystic fibrosis syndrome, since it stimulates pancreatic secretion.[3,4] However, slaframine has thus far only been produced in low yield, by surface cultures of *R. leguminicola*;[5] thus, difficulty in obtaining the metabolite from its natural source has limited its study and made its chemical synthesis an attractive prospect.

Structure **1** has been assigned to slaframine.[6] We should like to report here the preparation of racemic *cis,cis*-1-acetoxy-6-acetamidoindolizidine (**2**) and its identity with *N*-acetylslaframine, prepared earlier[1,6] from the natural compound. We also report the conversion of *N*-acetylslaframine to slaframine. Thus, the present report describes the total synthesis of slaframine.

The route chosen for the synthesis proceeded from 2-bromo-5 nitropyridine (**3**) (prepared from 2-hydroxy-5-nitropyridine by the method of Binz and Schickh,)[7] which was converted to 5-acetamido-2-carbethoxypyridine (**4**) by a modification of the route of Schmidt-Thomé and Goebel.[8] Fusion of **3** with cuprous cyanide and acidic hydrolysis of the resulting nitrile gave the carboxylic acid **5**,[9] mp 211–212° (lit.[8] 211–212°), which

(1) S. D. Aust, H. P. Broquist, and K. L. Rinehart, Jr., *J. Amer. Chem. Soc.*, **88**, 2879 (1966).
(2) J. H. Byers and H. P. Broquist, *J. Dairy Sci.*, **43**, 873 (1960); **44**, 1179 (1961).
(3) S. D. Aust, *Biochem. Pharmacol.*, **18**, 929 (1969).
(4) T. E. Spike, M.S. Thesis, Michigan State University, 1969.
(5) S. D. Aust, Ph.D. Thesis, University of Illinois, 1965.
(6) R. A. Gardiner, K. L. Rinehart, Jr., J. J. Snyder, and H. P. Broquist, *J. Amer. Chem. Soc.*, **90**, 5639 (1968).
(7) A. Binz and O. von Schickh, *Chem. Ber.*, **68**, 315 (1935).
(8) J. Schmidt-Thomé and H. Goebel, *Z. Physiol. Chem.*, **288**, 237 (1951).

was esterified in ethanol–sulfuric acid to **6**, mp 107–108°,[9,10a] in 11% overall yield (from **6** to **8**). Reduction of **6** over platinum oxide in ethanol gave the amino ester **7** (98% yield),[9,10a] mp 129–130° (lit.[8] 131–132°); acetylation of **7** with acetic anhydride gave the acetyl derivative **4** (76%), which crystallized from ethyl acetate as white needles, mp 156–157°.[9,11]

Hydrogenation of the pyridine ring at 50 psi[12] converted **4** to **8** (ethyl 5-acetamidopipecolate, 91% yield), a clear oil, bp 155–160° (0.060 mm).[9,10a] Heating **8** with ethyl acrylate in ethanol for 2 days[13] gave the diester **9** as a viscous oil (40%): bp 187–200° (0.05 mm);[9,10a] nmr ($CDCl_3$) $(-COOCH_2CH_3)_2$ at δ 1.28 (6 H, t, J = 7 Hz), 4.17 (2 H, q), and 4.11 (2 H, q). Cyclization of **9**, using potassium *tert*-butoxide in toluene at 0°,[14] gave the unstable β-ketoester **10**[10a] in 75% yield: ir ($CHCl_3$) 1760 (cyclopentanone), 1718 (ester), and 1655 cm^{-1} (amide); nmr ($CDCl_3$) $COOCH_2CH_3$ at δ 1.28 (3 H, t, J = 7 Hz) and 4.20 (2 H, q), $NCOCH_3$ at δ 1.96 and 1.94 (total 3 H, s). The latter two peaks indicated a mixture of isomers.

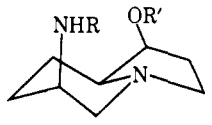
The β-ketoester (**10**) was hydrolyzed and decarboxylated by heating with 8 *N* hydrochloric acid at 100° for 5.5 hr[15] to give the ketone **11**,[10a] ir ($CHCl_3$) 1750 cm^{-1} (cyclopentanone). Reduction of **11** with sodium borohydride gave the important amino alcohol intermediate **12** (a mixture of stereoisomers, including **13**)[10a] in 30% yield from **10**, ir ($CHCl_3$) 3250 cm^{-1}. The mixture of amino alcohols (whose infrared spectrum lacked carbonyl absorption) was acetylated with acetic anhydride to give 1-acetoxy-6-acetamidoindolizidine (**14**)[10a] (81% yield), also as a mixture of stereoisomers. Careful chromatography on alumina separated the four stereoisomers, labeled A, B, C, and D, in order of their elution with chloroform: the approximate ratio of isomers isolated was 1:1.4:4:5 (A:B:C:D). The stereoisomers all had very similar mass spectra with peaks at m/e 240 (P), 181 (P − H_2NAc), and 121 (P − H_2NAc − HOAc). Isomer C was identical with *N*-acetylslaframine (**2**) by infrared spectrum, nmr spectrum, R_f on tlc, and retention time on glc. Isomers A, B, and D were differentiated from *N*-acetylslaframine by infrared and nmr spectra, glc, and tlc. The nmr spectra (Table I) of the four stereoisomers confirm the previous assignment of stereochemistry (**2**) to *N*-acetylslaframine (cis 1-H,8a-H, equatorial 6-H) and assign stereochemistry to the other isomers as follows: A, trans 1-H,8a-H, axial 6-H; B, trans 1-H,8a-H, equatorial 6-H; D, cis 1-H,8a-H, axial 6-H.

Deacetylation of **2** was achieved by boiling with hydrazine hydrate for 3 days.[16] The product, deacetylslaframine (**13**),[9,10b] was converted with benzyl chloroformate to *N*-carbobenzoxyslaframine (**15**), which was purified by chromatography on alumina (50% yield from *N*-acetylslaframine) and crystallized from ether as small white needles: mp 154–156°;[10] ir (KBr) 1695 cm^{-1} (carbamate); nmr ($CDCl_3$), δ 5.08 (2 H, s, $ArCH_2O$–CO) and 7.29 (5 H, s, C_6H_5). Acetylation with acetic anhydride gave the acetate **16** [93% yield; ir ($CHCl_3$) 1740 (ester) and 1690 cm^{-1}; nmr ($CDCl_3$), δ 1.98 (3 H, s, $COCH_3$)],[10] which was hydrolyzed to slaframine (**1**) in 98% yield by stirring with 30% hydrobromic acid in acetic acid for 1 hr.[17] Infrared, nmr, and mass spectra, as well as tlc and glc behavior of the slaframine obtained were identical with those of the natural product.

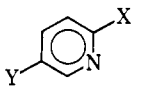
Table I. Nmr Absorption of 1-H and 6-H in 1-Acetoxy-6-acetamidoindolizidine Isomers

Isomer	Stereochemistry 1-H, 8a-H	6-H	1-H δ, ppm	1-H $W_{1/2}$, Hz	6-H δ, ppm	6-H $W_{1/2}$, Hz
A[a]	Trans	Axial	4.84	20		
B	Trans	Equatorial	4.76	21	4.25	9
C	Cis	Equatorial	5.31	13	4.24	8
D	Cis	Axial	5.32	14	4.12	20

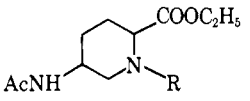
[a] The very small amount of isomer A isolated did not allow determination of δ or $W_{1/2}$ for H-6.



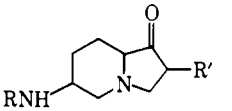
1, R = H; R' = Ac
2, R = R' = Ac
13, R = R' = H
15, R = Cbz; R' = H
16, R = Cbz; R' = Ac



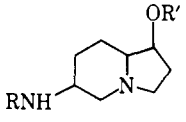
3, X = Br; Y = NO_2
4, X = $COOC_2H_5$; Y = NHAc
5, X = COOH; Y = NO_2
6, X = $COOC_2H_5$; Y = NO_2
7, X = $COOC_2H_5$; Y = NH_2



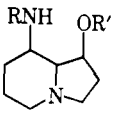
8, R = H
9, R = $-CH_2CH_2COOC_2H_5$



10, R = Ac; R' = $COOC_2H_5$
11, R = R' = H



12, R = R' = H
14, R = R' = Ac



17, R = H; R' = Ac
18, R = R' = Ac

Prior to assigning structure **1** to slaframine, we had earlier assigned it structure **17**.[1,18] In connection with the present work we undertook a synthesis of 1-acetoxy-8-acetamidoindolizidine (**18**) as well, beginning with 3-amino-2-carbethoxypyridine[19–21] and proceeding by a

(9) Acceptable microanalyses were obtained.
(10) The molecular weight was substantiated by (a) low-resolution mass spectrometry, (b) high-resolution mass spectrometry.
(11) Schmidt-Thomé and Goebel[8] reported mp 220–221° for **10**. However, the spectral data for our compound allow no doubts of its structure: ir (KBr) 1718 (ester) and 1690 cm^{-1} (amide); nmr ($CDCl_3$) $COOCH_2CH_3$ at δ 1.41 (3 H, t, J = 7 Hz) and 4.43 (2H, q), $NCOCH_3$ at δ 2.23 (3 H, s), H-3 at δ 8.04 (1 H, d, $J_{3,6}$ = 0, $J_{3,4}$ = 8.5 Hz), H-4 at δ 8.44 (1 H, q, $J_{4,6}$ = 2 Hz), H-6 at δ 8.72 (1 H, d), NH at δ 9.18 (1H, b).
(12) G. R. Clemo, N. Fletcher, G. R. Newton, and R. Raper, *J. Chem. Soc.*, 1140 (1950).
(13) N. J. Leonard, S. S. Swann, Jr., and J. Figueras, Jr., *J. Amer. Chem. Soc.*, 74, 4620 (1952).
(14) J. Blake, C. D. Wilson, and H. Rapoport, *ibid.*, 86, 5293 (1964).
(15) G. R. Clemo and G. R. Ramage, *J. Chem. Soc.*, 2969 (1932).
(16) W. Schroeder, B. Bannister, and H. Hoeksema, *J. Amer. Chem. Soc.*, 89, 2448 (1967).
(17) D. Ben-Ishai and A. Berger, *J. Biol. Chem.*, 17, 1564 (1952).
(18) After our earlier assignment[1] a second group also assigned the same incorrect structure (**17**) to slaframine: B. J. Whitlock, D. P. Rainey, N. V. Riggs, and F. M. Strong, *Tetrahedron Lett.*, 3819 (1966).
(19) E. Sucharda, *Chem. Ber.*, 58, 1727 (1925).
(20) V. Oakes, R. Pascoe, and H. N. Rydon, *J. Chem. Soc.*, 1045 (1956).
(21) L. R. Fibel and P. E. Spoerri, *J. Amer. Chem. Soc.*, 70, 3908 (1948).

sequence very similar to that described above.[22] The diacetate (**18**, presumably a mixture of stereoisomers but not separated on tlc or glc) gave spectral data very similar to those of *N*-acetylslaframine but was clearly different in both tlc and glc behavior.

Acknowledgment. This work was supported in part by Public Health Service Grant No. AI-04769 from the National Institute of Allergy and Infectious Diseases.

(22) Satisfactory spectral data, plus microanalyses or mass spectra, were obtained for all intermediates in this reaction sequence.

(23) Public Health Service Predoctoral Fellow and Allied Chemical Co. Fellow.

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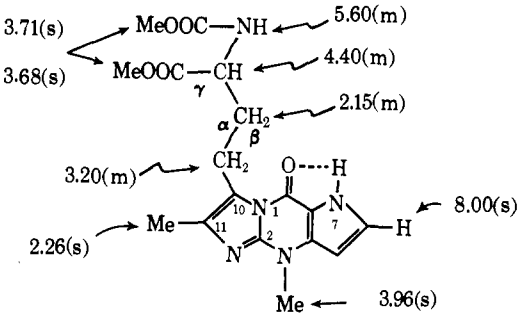
Structure of the Fluorescent Y Base from Yeast Phenylalanine Transfer Ribonucleic Acid

Sir:

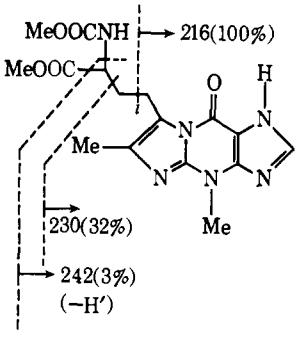
The tRNA's from different organisms contain numerous odd bases. Especially interesting are the tRNA's with a 3′-terminal A in their anticodons, which in turn are linked to N^6-isopentenyladenosine (*i*-A) or 2-methylthio-*i*-A.[1-3] The phenylalanine tRNA of yeast,[4] wheat germ,[5] and rat liver[6] contain a fluorescent base Y of unknown structure adjacent to the A at the 3′ end of the anticodon. The Y base linked to the anticodon, 2′-OMeGAA, in phenylalanine tRNA of yeast ($tRNA_{yeast}^{Phe}$)[4] has attracted great interest because of its important biochemical role and intense fluorescence.

Mild acid treatment of $tRNA_{yeast}^{Phe}$ splits off the Y base without breaking the tRNA chain.[7] The acid-treated tRNA ($tRNA_{HCl}^{Phe}$) thus obtained can still be charged with Phe to give Phe-$tRNA_{HCl}^{Phe}$, but the coding properties of the latter are significantly modified.[7,8] The Y base fluorescence has been utilized in tRNA tertiary structural studies,[9] and luminescence studies indicate that the phosphorescence spectrum of $tRNA_{yeast}^{Phe}$ is similar to guanines.[10]

In spite of the efforts of several groups to elucidate its structure, this has still remained unsolved due to scarcity of material and structural complexity. We propose structure **1** (and **2**) for Y base[11] from spectral data



1



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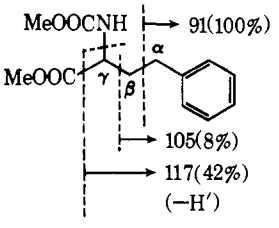

3

obtained with 300 μg of material and synthetic models.

Isolation. The procedure was essentially that of Thiebe and Zachau.[7] Crude $tRNA^{Phe}$ (400 mg) was separated from 15 g of yeast tRNA mixture by a benzoylated DEAE-cellulose column and the $tRNA^{Phe}$, after incubation at 37° for 3–5 hr at pH 2.9, was extracted with ethyl acetate[12] (instead of chloroform[7]). The extracted crude Y base (500 μg) was applied to cellulose tlc, using the upper phase of ethyl acetate–1-propanol–water (4:1:2), and the strongly fluorescent band with R_f 0.77[13] was eluted with water-saturated ethyl acetate[12] to yield 300 μg of amorphous material.[14]

The uv, ir,[15] pK_a', nmr,[16] and CD data for **1** are: uv (10% MeOH) 235 (ε 23,500), 263 (4500), 313 nm (3500); uv (10% MeOH, pH 2.1) 235 (ε 22,800), 285 nm (4615); uv (10% MeOH, pH 9.4) 236 (ε 23,500), 264 (4400) 303 nm (4350); ir (KBr) 1725 (COOMe), 1715 (COOMe and nuclear CO), 1600 cm^{-1}; pK_a' (10% MeOH) 3.22 ± 0.10, 7.42 ± 0.10 (from uv); CD (10% MeOH) $\Delta\epsilon_{235}$ −2.2 ± 0.3; $\Delta\epsilon_{260}$ −0.5 ± 0.3. The mass spectral data[17,18] for **2** are: calcd for $C_{16}H_{20}N_6O_5$, 376.1495; found M^+ 376.1497[17a] (37%); M − MeOH 344 (5%). The nmr[16] showed four Me singlets, one aromatic H, one D-exchangeable H (5.60 ppm), and a set of three signals, which in conjunction with the mass spectral base peak at *m/e* 216 (see **2**),

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(3) W. J. Burrows, D. J. Armstrong, F. Skoog, S. M. Hecht, J. T. A. Boyle, N. J. Leonard, and J. Occolowitz, *ibid.*, **161**, 691 (1968); F. Harada, H. J. Gross, F. Kimura, S. H. Chang, S. Nishimura, and U. L. RajBhandary, *Biochem. Biophys. Res. Commun.*, **33**, 299 (1968).

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(9) K. Beardsley and C. R. Cantor, *Proc. Nat. Acad. Sci. U. S.*, **65**, 39 (1970).

(10) J. Eisinger, B. Feuer, and T. Yamane, *ibid.*, **65**, 638 (1970).

(11) For the sake of convenience, the numbering system is based on guanine.

(12) Ethyl acetate was used because the Y base appeared to be rather unstable when left in chloroform for some period.

(13) Another minor bond at R_f 0.95 was present on tlc, but this was not pursued further because of its very weak fluorescence and extremely minute quantity.

(14) As judged from ε values of model **11**, the Y base purity is *ca.* 90%.

(15) A micro-ir was also measured by Mr. W. F. Fulmor, Lederle Laboratories, Pearl River, N. Y., to whom we extend our gratitude.

(16) We acknowledge Mr. R. Pitcher, Hoffmann-La Roche, for the spectrum measured with a Varian HA-100/CAT system (36 scans).

(17) (a) MS9, 70 eV, 165°; we thank Dr. G. Van Lear, Lederle Laboratories, for these measurements and discussion. (b) CEC21-110-B; 25 and 75 eV, 150°, Columbia University. All peaks stronger than 1% relative intensity were measured by high-resolution techniques.

(18) Per cents in parentheses in **2** and **3** are relative intensities in low-resolution mass spectra.