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.

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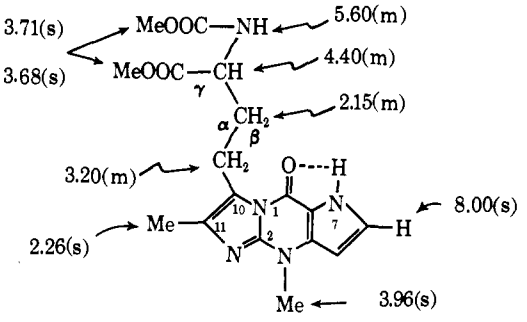
Structure of the Fluorescent Y Base from Yeast Phenylalanine Transfer Ribonucleic Acid

Sir:

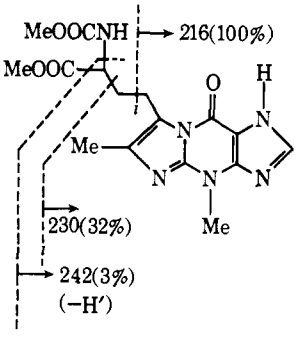
The tRNA's from different organisms contain numerous odd bases. Especially interesting are the tRNA's with a 3′-terminal A in their anticodons, which in turn are linked to N^6-isopentenyladenosine (*i*-A) or 2-methylthio-*i*-A.[1-3] The phenylalanine tRNA of yeast,[4] wheat germ,[5] and rat liver[6] 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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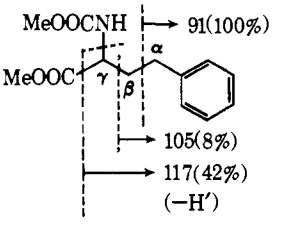


3

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**),

(1) H. G. Zachau, D. Dutting, and H. Feldmann, *Z. Phys. Chem.*, 347, 2121 (1966).

(2) J. T. Madison, G. A. Everett, and H. Kung, *Science*, **153**, 531 (1966).

(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).

(4) U. L. RajBhandary, S. H. Chang, A. Stuart, R. D. Faulkner, R. M. Hoskinson, and H. G. Khorana, *Proc. Nat. Acad. Sci. U. S.*, **57**, 751 (1967).

(5) B. S. Dudock, G. Katz, E. K. Taylor, and R. W. Holley, *ibid.*, 62, 941 (1969).

(6) L. M. Fink, T. Goto, F. Frankel, and I. B. Weinstein, *Biochem. Biophys. Res. Commun.*, 32, 963 (1968).

(7) R. Thiebe and H. G. Zachau, *Eur. J. Biochem.*, **5**, 546 (1968).

(8) K. Ghosh and H. P. Ghosh, *Biochem. Biophys. Res. Commun.*, 40, 135 (1970).

(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.

were assignable to a system such as $-CHCH_2CH_2-$ attached to an aromatic nucleus. One H could not be detected in the nmr probably due to broadening or overlap.

Side Chain. The *m/e* 216 base peak and possible presence of a $-CHCH_2CH_2-$ (aromatic) group suggested that the side chain and nucleus consisted of $C_7H_{12}NO_4$ and $C_9H_8N_5O$ moieties, respectively. The presence of two weak CD Cotton effects at wavelengths corresponding to the nuclear uv absorptions further suggested that the side-chain methine carbon was the chiral center, and that the weak CD extrema were due to slight perturbation of the aromatic absorptions by the distant chiral center. Moreover, the CD curves remained unchanged when solutions were maintained at pH 3 and 9 for 24 hr, an observation which excluded any possibility of a readily enolizable group being attached to the chiral methine.

In view of this the side-chain model *dl*-**3** was synthesized (phenethyl bromide to homophenylalanine, then esterification and carbamation): mass spectrum M^+ 251 (1%); M − MeOH, 219 (1%); ir (film) 1730 cm^{-1}; nmr ($CDCl_3$) α-CH_2, 2.68 (m); β-CH_2, 2.11 (m); γ-CH, 4.39 (m); NH, 5.40 (br d); COOMe, 3.70 and 3.72 ppm. As shown for **1**, **2**, and **3**, all spectral data are in full agreement, the only difference being the chemical shifts of α-CH_2 groups (3.20 *vs.* 2.68 ppm), which is expected. The side chain in Y is thus established.

Nucleus. A critical observation was that when the methanol solution of Y was injected into the mass spectral inlet system,[17b] the spectrum showed clear peaks at *m/e* 390, 358, 256, 244, and 230, in addition to lower original Y peaks located at *m/e* 14. This could only be accounted for by the type of conversion in which a nuclear "carbonyl group" **4** is transformed into its enol ether **5**. The $C_9H_8N_5O$ nucleus thus should bear olefinic (2.26 ppm) and *N*-methyl (3.96 ppm) groups and a "carbonyl group."

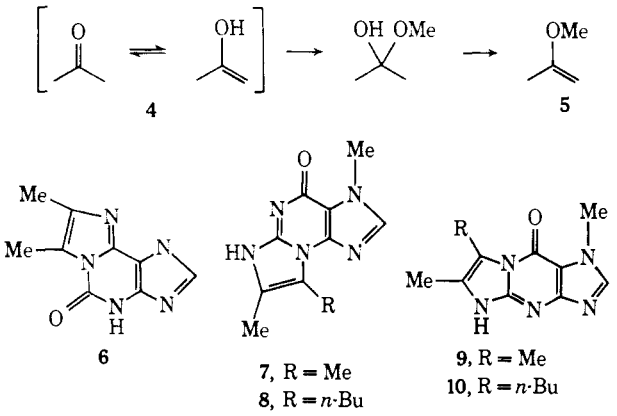
Since the presence of an N^7-methyl group is well known to labilize the ribose residue to acids,[19] models **6**–**10** were synthesized by unequivocal methods.[20] The uv spectra of **6**–**8** clearly ruled out their possibilities as being the Y nucleus: **6**, uv (10% MeOH) 222 (ϵ 8400), 297 (13,900), 305 (14,300), 317 nm (sh, 8600); **8**, uv (10% MeOH) 225 (ϵ 23,700), 277 nm (9890); **10**, uv (10% MeOH) 232 (ϵ 25,600), 260 (sh, 3030), 305 nm (5600); uv (10% MeOH, pH 2.0) 230 (ϵ 17,500), 290 nm (broad, 4200); uv (10% MeOH, pH 12.0) 235 (ϵ 25,600), 265 (4690), 330 nm (4610). However, the maxima and shapes of neutral and acid uv of **9** and **10** were sufficiently similar to those of Y to suggest that the difference in nuclear structures was probably the position of the *N*-methyl group.

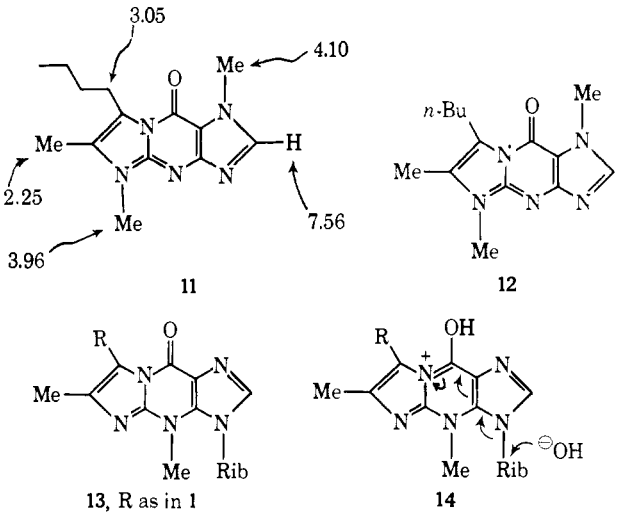
The tricyclic guanine derivative **10** was therefore methylated to give **11** (major product) and **12** (minor product) having fixed double bond structures. Spectral data for **11** and **12** are: **11**, uv (10% MeOH) 237 (ϵ 26,000), 265 (4700), 315 nm (3850); uv (10% MeOH, pH 2.1) 237 (ϵ 23,600), 292 nm (5850); ir (KBr) 1720, 1600, 1595 cm^{-1}; for **12**, uv (10% MeOH) 235 (ϵ 26,300), 265 (sh, 4250), 313 nm (5410); uv (10% MeOH, pH 2.1) 233 (ϵ 18,560), 277 (5660), 305 nm (4000). Compound **12** which was synthesized by an alternative unambiguous route (base condensation of 2-methylamino-N^7-methylinosine and 3-bromoheptan-2-one),[20] exhibited an acid uv spectrum (λ_{max} 233, 277, 305 nm) clearly different from the Y base spectrum. In contrast, the neutral and acid uv of **11** were almost identical with those of Y excepting for the uniformly smaller ϵ values in Y;[14] also the nmr peaks were in excellent agreement (compare **1** and **11**).

As it is well established that substitution of N–H for N–Me has only a minor influence on the uv spectra of N-heteroaromatic systems in which the double bond arrangements are identical, the Y base *nucleus* should be as represented in **1** (the N^7–H tautomer presumably being stabilized by H bonding). The fact that the Y side chain and olefinic methyl group are attached to C-10 and C-11, respectively, is established from the following data. The olefinic methyl in model **10** has its nmr speak at 2.25 ppm; therefore the two *C*-methyl nmr peaks in **9**, which appear at 2.61 and 2.25 ppm, are assignable to the 10-Me and 11-Me, respectively. As the olefinic methyl peak of Y is at 2.26 ppm, the methyl must be bonded to C-11.

Biosynthetically Y base may be regarded as a guanine modified in the following manner. Thus, C_1 and C_2 units are attached, respectively, to N^3 and the C-2 amino group, a glutaric acid residue is attached to N^1, and cyclization occurs between C-10 and C-11. The glutaric acid amino group is transformed into a carbamate at some stage.

The most straightforward representation for Y nucleoside based on **1** is **13**.[21] Facile cleavage of the





(19) J. A. Zoltewicz, D. F. Clark, T. W. Sharpless, and G. Grahe, *J. Amer. Chem. Soc.*, **92**, 1741 (1970).
(20) M. Funamizu, N. Furutachi, and K. Nakanishi, to be published.

Y-ribose bond by acid can be accounted for by participation of the protonated canonical structure **14**, which leads to irreversible liberation of the Y base.

The proposed structure of Y base represents the most complex modified base that has been found thus far in any RNA molecule. This unusual degree of modification may be necessary to stabilize the relatively weak codon–anticodon interaction expected for phenylalanine.

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(21) Isolation of Y nucleoside by enzymatic cleavage and direct structural studies of the nucleoside are essential for establishment of the nucleoside structure.

(22) Career Scientist of the Health Research Council of the City of New York (I-190).

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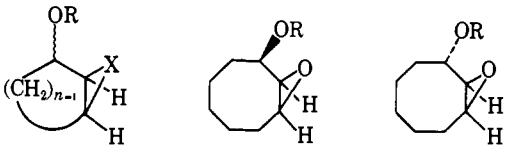
Solvolysis of *syn*- and *anti*-9-Oxabicyclo[6.1.0]non-2-yl *p*-Bromobenzenesulfonates

Sir:

The solvolytic reactions of *syn*- and *anti*-2-bicyclo[*n*.1.0]alkyl systems Ia have recently been the subject of extensive research.[1] The important effect that the stereochemistry of the leaving group has upon the product distributions is of particular interest. Whereas the solvolytic reaction products of Ia, with n = 2–5,[1a-e] have been shown to be independent of the orientation of the leaving group relative to the cyclopropane ring, other larger medium-ring cyclopropylcarbinyl systems yield one product mixture when the leaving group is syn to the cyclopropane ring, and a completely different product mixture when the leaving group is anti to the cyclopropane ring.[1f-h] We have therefore undertaken a study of the structurally related *syn*- and *anti*-oxabicyclo[*n*.1.0]alkyl systems Ib to make a comparison between the chemical reactivities of epoxycarbinyl and cyclopropylcarbinyl derivatives. We wish to report that this study has revealed a large syn:anti rate ratio and distinctly different product distributions for the solvolyses of *syn*- and *anti*-9-oxabicyclo[6.1.0]non-2-yl *p*-bromobenzenesulfonates (IIb[2] and IIIb[3]).



Ia, X = CH_2 b, X = O

IIa, R = H b, R = Bs

IIIa, R = H b, R = Bs

Products from hydrolysis of IIb and IIIb in 80% acetone–water are summarized in Table I. Of significance is the fact that the major products from hydrolysis of IIIb (namely IIIa, VI, and VIII[4]) are formed in only trace amounts from IIb. Also, the major products from IIb are isolated as relatively minor products from IIIb. The very different product distributions require that IIb and IIIb solvolyze through distinctly different mechanistic pathways, with a maximum of 1–2% crossover between pathways.

syn-*p*-Bromobenzenesulfonate IIb solvolyzed 259 times faster than IIIb at 25° (Table II), and yielded mainly suberaldehyde (V). A plausible mechanism that might account in part for the syn:anti rate ratio and large amount of V produced from IIb involves a competition between backside participation by the C–C bond of the oxirane ring (Scheme I) and solvent-assisted ionization. Such assistance of the oxirane ring would lead to oxonium ion XII, perhaps *via* an intermediate ion XI.[5]

Table I. Product Distributions from Hydrolysis of IIb and IIIb in 80% Acetone–Water[a]

Product	IIa	IIIa	V	VI	VII	VIII	IX
			HC(=O)$(CH_2)_3$C(=O)H	CHO	HO O	HO O	O
% from IIb	9.1	1.5	52.4	2.8	2.5[b]	~0[b]	28.3
% from IIIb	5.1	36.0	1.9	17.4[c]	~0[b]	28.9[b]	4.9

[a] Triethylamine was used as a buffer. [b] Epimers VII and VIII could not be separated on glpc. However, analysis of the ir spectra of the solvolysis compounds indicated the lack of contamination ($\lesssim$5%) of the other epimer.

(1) (a) K. Wiberg, V. Williams, Jr., and L. Friedrich, *J. Amer. Chem. Soc.*, 90, 5338 (1968); (b) E. C. Friedrich and S. Winstein, unpublished work; (c) H. L. Goering and K. E. Rubenstein, Abstracts, 151st National Meeting of the American Chemical Society, Pittsburgh, Pa., March 28–31, 1966, p 5K; (d) A. C. Cope and P. E. Peterson, *J. Amer. Chem. Soc.*, 81, 1643 (1959); (e) A. C. Cope, S. Moon, and P. E. Peterson, *ibid.*, 84, 1935 (1962); (f) M. Gasic, D. Whalen, B. Johnson, and S. Winstein, *ibid.*, 89, 6382 (1967); (g) D. Whalen, M. Gasic, B. Johnson, H. Jones, and S. Winstein, *ibid.*, 89, 6384 (1967); (h) C. D. Poulter and S. Winstein, *ibid.*, 92, 4274, 4282 (1970).

(2) Appropriate spectral data and elemental analyses were obtained: mp of IIb, 117.5–118.5°; mp of IIIb, 85.5–86.5°. *syn*-Alcohol IIa, mp 89.5–91.0°, was prepared by sodium borohydride reduction (99% stereospecificity) of 9-oxabicyclo[6.1.0]nonan-2-one.[3]

(3) A. C. Cope, A. Keough, P. Peterson, H. E. Simmons, Jr., and G. Wood, *J. Amer. Chem. Soc.*, 79, 3900 (1957).

(4) Compounds VII and VIII were oxidized with Jones reagent to 9-oxabicyclo[6.1.0]nonan-3-one; ir (CCl_4) 1705 cm^{-1}.

(5) The reaction of X → XI is related to the cyclopropylcarbinyl–homoallyl rearrangement. The net retention of stereochemistry at C-2 might be explained by ion XI; see ref 1f and g.