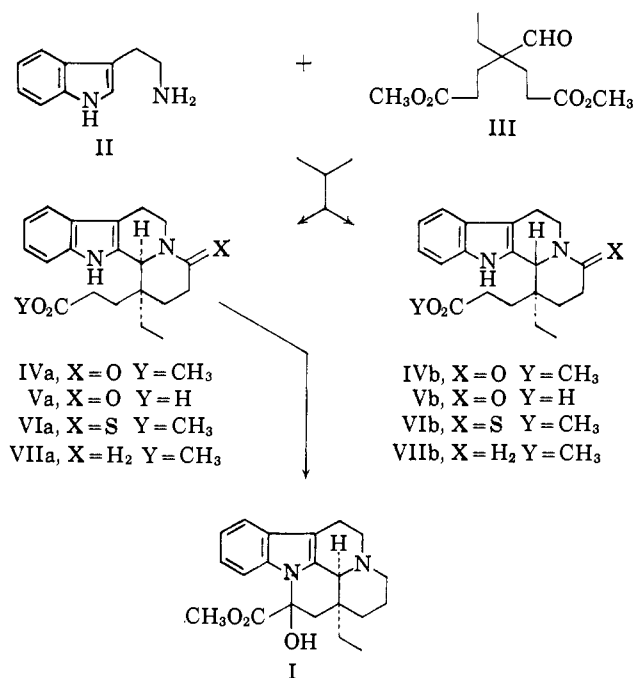## The Total Synthesis of Vincamine

Sir:

Vincamine, the antihypertensive and sedative[1] major alkaloid of *Vinca minor* L. (Apocyanaceae) was first isolated and characterized by Schlittler.[2] Recent degradative experiments[3-8] have established structure I for this compound, with the stereochemistry of the D–E ring junction related to the previously synthesized alkaloid eburnamonine.[9,10] The synthesis of vincamine has now been accomplished starting from tryptamine (II), which was condensed with dimethyl 3-ethyl-3-formylpimelate (III), b.p. 108–109° (0.03 mm.). *Anal.* Calcd. for $C_{12}H_{20}O_5$: C, 59.00; H, 8.25. Found: C, 58.88; H, 8.20. The latter was readily obtained by exhaustive alkylation of the pyrrolidine enamine of butyraldehyde[11] with methyl acrylate, followed by hydrolysis (45% yield).

IVa, X = O Y = CH₃
Va, X = O Y = H
VIa, X = S Y = CH₃
VIIa, X = H₂ Y = CH₃

IVb, X = O Y = CH₃
Vb, X = O Y = H
VIb, X = S Y = CH₃
VIIb, X = H₂ Y = CH₃

The key intermediate lactam ester IVa and its epimer IVb were isolated as a crystalline mixture (53% yield), m.p. 160–182°. *Anal.* Calcd. for $C_{21}H_{26}N_2O_3$: C, 71.17; H, 7.39; N, 7.90. Found: C, 70.89; H, 7.46; N, 7.59. This was accompanied by a mixture of crystalline lactam acids Va and Vb (37% yield), m.p. 226–240°. *Anal.* Calcd. for $C_{20}H_{24}N_2O_3$: C, 70.57; H, 7.11; N, 8.23. Found: C, 70.56; H, 7.12; N, 8.16. Hydrolysis of the lactam esters IVa and IVb

with aqueous sodium bicarbonate furnished the lactam acids Va and Vb (quantitative yield), which could be converted back to the methyl esters with diazomethane (quantitative yield). Reaction of the mixture of epimeric lactam esters IVa and IVb with phosphorus pentasulfide gave the individual epimeric thiolactam esters VIa (29% yield), m.p. 163–164°, and VIb (29% yield), m.p. 144–145°, which were readily separated by column chromatography on neutral alumina. *Anal.* Calcd. for $C_{21}H_{26}N_2O_2S$ (VIa): C, 68.07; H, 7.07; N, 7.56; S, 8.66. Found: C, 67.84; H, 6.95; N, 7.38; S, 8.94. *Anal.* Calcd. for $C_{21}H_{26}N_2O_2S$ (VIb): C, 68.07; H, 7.07; N, 7.56; S, 8.66. Found: C, 68.05; H, 7.09; N, 7.42; S, 8.77. Desulfurization of the thiolactams with Raney nickel furnished the amino esters VIIa (54% yield), m.p. 149–150°, and VIIb (54% yield), m.p. 144–145°. *Anal.* Calcd. for $C_{21}H_{28}N_2O_2$ (VIIa): C, 74.09; H, 8.29. Found: C, 74.13; H, 8.32. *Anal.* Calcd. for $C_{21}H_{28}N_2O_2$ (VIIb): C, 74.09; H, 8.29. Found: C, 74.20; H, 8.40.

Alternatively, the epimeric mixture of thiolactams could be converted to a mixture of amino esters and this then separated by column chromatography on neutral alumina. By either route one obtained the epimeric compounds in equal amounts. Mercuric acetate oxidation of either amino ester VIIa or VIIb to a didehydro immonium salt with ultraviolet absorption at 250 and 359 mμ and reduction of the immonium salt with sodium borohydride gave a mixture of amino esters VIIa and VIIb. Thus a path is provided for complete conversion of intermediates to either of the epimeric series.

Oxidation of the more rapidly eluted amino ester VIIa with *p*-nitrosodimethylaniline and triphenylmethylsodium, followed by carefully controlled acid treatment, furnished *dl*-vincamine (I, 3% yield), which was identified by comparison with an authentic sample of natural vincamine by exact matching of t.l.c. retention times in multiple adsorption and solvent systems and by infrared solution spectra.

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Studies on the Azidoazomethine–Tetrazole Equilibrium[1]

Sir:

Treatment of 5-aminotetrazole with acetylacetone[2a] and reaction of 4,6-dimethyl-2-hydrazinopyrimidine with nitrous acid[2b,c] have been reported to give the same product, 5,7-dimethyltetrazolo[1,5-*a*]pyrimidine (IA).[3]

(1) This investigation was supported by funds from the C. F. Kettering Foundation and the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. PH-43-64-51.

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TABLE I

EQUILIBRIUM CONSTANTS AND PROTON MAGNETIC RESONANCE SPECTRA ASSIGNMENTS[a]

| | | | r-Value (multiplicity) $(J$ in c.p.s.)[b] | | | | |
| | | | Tetrazolo[1,5-a]pyrimidine | | | Azidopyrimidine | |
Solvent	% Concn. (w./v.)	K_T[d]	5-CH₃	7-CH₃	6-H	4(6)-CH₃	5-H
DMSO-d₆[c]	5	e	7.31 (s)	7.11 (d)(1.00)	2.62 (q)	...	...
TFAA[c]	10	e	...	...	...	7.23 (d)(0.55)	2.65 (m) /
Pyridine	5	0.14	7.43 (s)	7.25 (d)(1.00)	/	7.72 (d)(0.50)	/
CDCl₃	7	0.36	7.22 (s)	7.00 (d)(1.10)	2.92 (q)	7.53 (d)(0.50)	3.15 (m)
Acetone-d₆	5	0.08	7.28 (s)	7.03 (d)(0.90)	2.65 (q)	7.58 (d)(0.55)	2.95 (m)

[a] Spectra were obtained on a Varian A-60 spectrometer using tetramethylsilane as internal standard. [b] s = singlet, d = doublet, q = quartet, m = multiplet. Precision in J = ±0.05 c.p.s. [c] DMSO-d₆ = dimethyl sulfoxide-d₆, TFAA = trifluoroacetic acid. [d] Ratio of the integrated intensities of the methyl signal from IB to the sum of methyl signals from IA at ambient temperatures (37–38°). The estimated mean deviation in K_T was less than ±0.02. [e] Only one tautomer was detected. [f] Position uncertain because of solvent interference.

The transparency of the solid-state infrared spectrum of I[4a] in the azido absorption region (2160–2120 cm.⁻¹)[4b] provides support for the assigned tetrazolo[1,5-a]pyrimidine structure. We have observed, however, that in solution I can exist either as the tetrazolo[1,5-a]pyrimidine (IA) or as the 2-azidopyrimidine (IB).

(structure IA) ⇌ (structure IB)
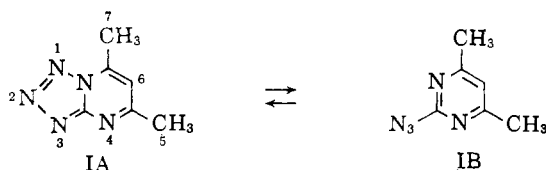
Existence of an azidoazomethine–tetrazole equilibrium has been demonstrated for at least two heterocyclic systems.[5a] Heretofore the cause for destabilization of the more stable tetrazole tautomer in fused-ring tetrazoles has been attributed to electron withdrawal which also results in stabilization of the electron-donating azido group.[5] During our investigation of this equilibrium we have found solvent and temperature effects which we believe to be new and significant. Azido absorption is absent in the infrared spectrum[8] of a N,N-dimethylformamide solution of I, but present at 2180 cm.⁻¹ in the spectrum of a trifluoroacetic acid solution.[6] Further, the p.m.r. spectra[7] of I indicate the presence of only the tetrazolo tautomer (IA) in dimethyl sulfoxide-d₆ and only the azido tautomer (IB) in trifluoroacetic acid. These assignments are substantiated by the characteristic, solvent independent pattern of the methyl group bands. In the azido tautomer (IB) the methyl groups are equivalent and were found to be involved in long-range coupling with the 5-proton. The methyl groups of the tetrazolo tautomer (IA), however, are nonequivalent and only the less shielded methyl group, no doubt in the 7-position, is coupled with the ring proton (see Table I). The p.m.r. spectra show that I in other solutions exists as a tautomeric mixture of the tetrazolo and azido forms (see Fig. 1). For each of these solutions an equilibrium
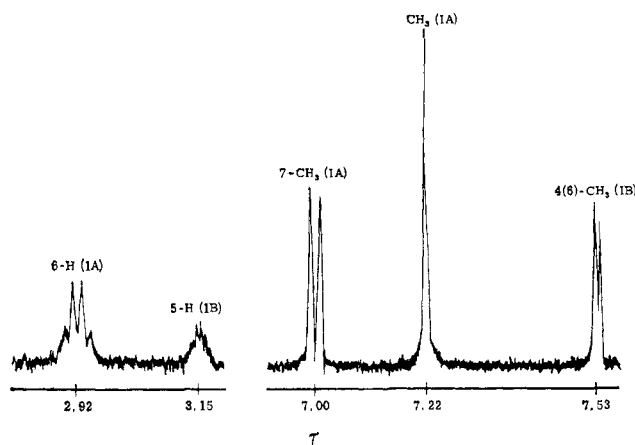

Fig. 1.—P.m.r. spectrum of I in deuteriochloroform measured at 60 Mc. from tetramethylsilane as an internal standard.

constant (K_T) was calculated from the ratio of the integrated intensities of the methyl signal from IB to the sum of the methyl signals from IA (see Table I). Apparently this is the first instance in which the relative amounts of the tetrazolo and azido tautomers have been determined.

Undoubtedly protonation of a pyrimidine nitrogen of IA occurs in trifluoroacetic acid, which results in electron withdrawal from the tetrazole ring and gives rise to IB. In contrast, the effect of a basic medium (pyridine) on tetrazole ring destabilization in IA is small. The variation of the equilibrium constant of I in the aprotic solvents implies that the ratio of the solubilities of IB to IA decreases as the polarity of the solvent increases.[8] In support of this observation dilution of a 6.8% solution of I in deuteriochloroform to 5.4% with dimethyl sulfoxide-d₆ results in a change of K_T from 0.36 to 0.10. Although dilution of a second solution with additional deuteriochloroform appeared to increase the concentration of IB relative to IA, the limited solubility of I did not allow us to obtain conclusive evidence for a concentration effect. Finally, the increase in the equilibrium constant[9] with temperature in deuteriochloroform is well correlated (see Fig. 2, correlation coefficient = 0.994) with a log K_T vs. $1/T$ relationship, which also shows that the tautomerization is endothermic (ΔH = +6.8 ± 0.5 kcal.-mole⁻¹).

(4) (a) The infrared spectra were determined in pressed potassium bromide disks or, when indicated, in solution with a Perkin-Elmer Model 221-G spectrophotometer. (b) I., J. Bellamy, "The Infrared Spectra of Complex Molecules," 1st Ed., John Wiley and Sons, Inc., New York, N. Y., 1954, p. 223.

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(6) The infrared spectrum of the trifluoroacetic acid solution was determined in an Irtran-2, fixed-thickness cell.

(7) The authors are indebted to Dr. W. C. Coburn and Mrs. Martha C. Thorpe for their aid in the interpretation and treatment of the p.m.r. spectra.

(8) G. W. Wheland, "Advanced Organic Chemistry," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1949, p. 607.

(9) The equilibrium constants were calculated from p.m.r. spectra determined on a Varian A-60 spectrometer by Dr. Ross G. Pitcher, Varian Associates, Palo Alto, Calif. Each determination was the mean of three runs at the specified temperature, which was verified by the chemical shift difference of the ethylene glycol peaks. The least square standard deviation in K_T was less than ±0.01.
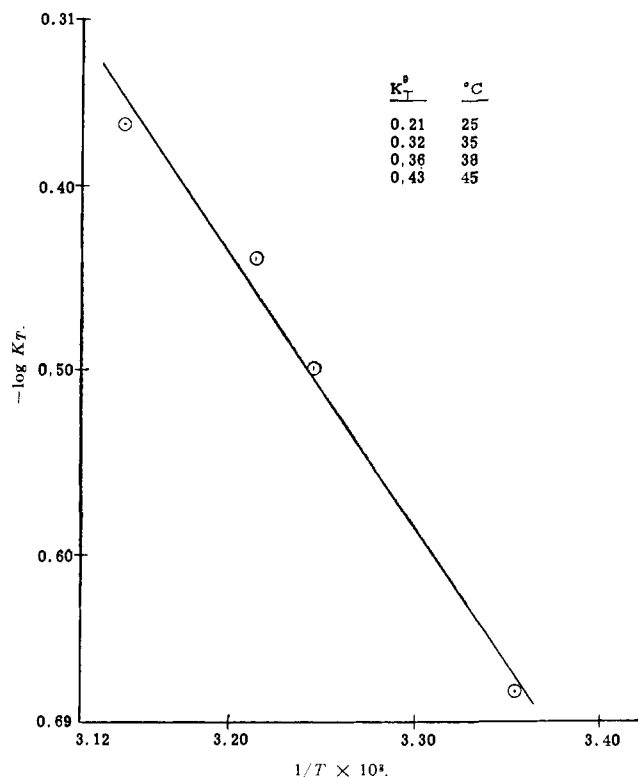
K_T^9	°C
0.21	25
0.32	35
0.36	38
0.43	45

$1/T \times 10^3$.

Fig. 2. Effect of temperature on the equilibrium constant (K_T) and the plot of log K_T vs. the reciprocal of the absolute temperature.

Additional information about I, together with the results of work on related compounds, will be published at a later date.

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Isolation of a 2-Nitropurine from the Reaction of Guanosine with Nitrous Acid

Sir:

It has been long recognized that nitrous acid reacts with those naturally occurring pyrimidines and purines that contain a primary amino group.[1] A great deal of interest has been focused on this reaction recently, with the discovery that nitrous acid, when applied to nucleic acids, is a potent mutagen.[2] Schuster, *et al.*,[3] have stated that the changes produced by nitrous acid within a molecule of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) were the same as those resulting from the action of nitrous acid upon the simple pyrimidine and purine bases or nucleosides: the amino groups of the adenine, cytosine, and guanine moieties were replaced by carbonyl groups. It was proposed that the first two changes led to mutations and that the third was lethal. More recently, however, various observations have been made which were not encompassed by this scheme. Several workers have noted a cross-linking effect upon the double helix of DNA produced

by nitrous acid.[4] The production of deletion mutations by this reagent has also been observed.[5] A synthetic polynucleotide containing uracil and guanine was found to suffer a pronounced loss of activity upon treatment with nitrous acid.[6] In a later communication, Schuster and Wilhelm[7] noted that all of the guanine of tobacco mosaic virus RNA was not being converted to xanthine, but that some was being consumed in an unknown reaction.

In order to obtain some knowledge as to the nature of these side reactions, we reinvestigated the reaction of nitrous acid with the ribonucleosides adenosine, cytosine, and guanosine. No product, other than the expected one, was observed with the first two nucleosides. The deamination of guanosine, however, in acetate buffer, led to the formation of a yellow side product in yields up to 5%. The highest yield was formed when a large excess of nitrite ion was used and the temperature was lowered to 0°. On the basis of the properties reported below, this product has been assigned the structure I, 2-nitroinosine (isolated as its

I
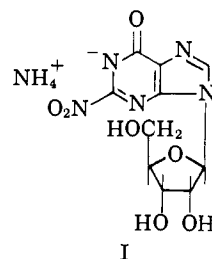
ammonium salt). I could be separated from xanthosine and any unreacted guanosine by paper chromatography (isobutyric acid–ammonia–water, 66:5:29) or, on a larger scale, by anion exchange chromatography on Amberlite CG 400 resin (acetate form). Because of its acidity (see below), 2-nitroinosine adhered firmly to the resin and was eluted with 0.1 N HCl and 1 N NaCl. It was recovered from that solution by adsorption onto charcoal and elution with ethanol–ammonia–water solution. Evaporation of the eluate gave I as a pale yellow powder. It recrystallized poorly, but an analytical sample could be prepared by repeated washing with cold 95% ethanol. *Anal.* Calcd. for $C_{10}H_{14}N_6O_7$: C, 36.37; H, 4.27; N, 25.45; O, 33.91. Found: C, 36.29; H, 4.40; N (by difference), 25.04; O, 34.27. I was titrated as an ammonium salt. *Anal.* Calcd. equiv. wt.: 330. Found: 320. I did not melt but decomposed when heated above 180°. It was destroyed by heating for 1 hr. in aqueous solution, pH 1.8, at 90°; ultraviolet spectrum: λ_{max}^{pH1} 222 (ϵ 12,000) and 335 mμ (4200); λ_{max}^{pH7} 233 (ϵ 14,400) and 343 mμ (3800). The infrared spectrum of I (KBr) showed a strong band at 6.20 μ (inosine, in alkaline solution, absorbs at 6.27 μ[8]) and bands at 6.40 and 7.38 μ which may be ascribed to the nitro function. Only one dissociation of 2-nitroinosine between pH 1 and 13 was detected by ultraviolet spectrophotometry. This dissociation was found to correspond to a pK_a of 3.3

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