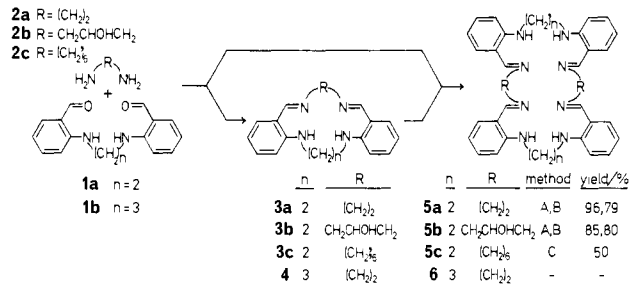
Scheme I[a]



[a] Reaction conditions are (A) 1a (2 mmol) + 2 (2.2 mmol) in MeOH (40–50 cm³) refluxed 6 h and a further 24 h after addition of $CHCl_3$ (5 cm³); (B) **3** heated 24 h in refluxing MeOH containing acetic acid (1 mol %); (C) **1a** (10 mmol) + **2c** (11.5 mmol) in EtOH (50 cm³) refluxed for 1.5 h, filtered, and set aside for 12 h.

collected without special precautions to protect the crystals from atmospheric moisture. Structure determination showed this material to be tetrabenz[*a,g,o,u*]-1,5,8,12,15,19,22,26-octaaza-29,30,31,32-tetraborapentacyclo[$1^{1,5}.1^{8,12}.1^{15,19}.1^{22,26}$]dotriacontane in which a B–H unit has been incorporated between each pair of *o*-iminoanilino nitrogen to give the unusual 20-membered B–N heterocycle shown in Figure 2. The two halves of the molecule are related by a crystallographic 2-fold axis perpendicular to the best plane through the inner great ring.

The isolation of the potentially octadentate macrocycles **5** and **8** presents the interesting possibility of preparing a series of dinuclear complexes in which the separation and disposition of the two metal ions is controlled by ring sizes and other geometric constraints in the ligands.

Acknowledgment. We thank the Science Research Council (U.K.) for studentships (to R.P., P.M.J., and K.P.D.) and diffractometer equipment and computing facilities. P.A.T. thanks the Regents of the University of California for support during a period of leave spent at the Irvine Campus.

Supplementary Material Available: Fractional coordinates, thermal parameters, bond distances, bond angles and observed and calculated structure factors for compounds **5b** and **7** (7 pages). Ordering information is given on any current masthead page.

Assignment of the Nitrogen-15 Nuclear Magnetic Resonances of Biotin and Unequivocal Synthesis Of (+)-[1-^{15}N]Biotin[1]

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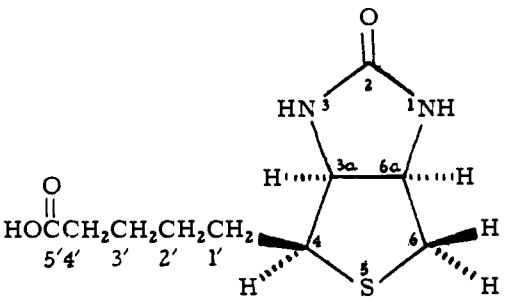
(+)-Biotin (**1**) is an essential cofactor for several enzymes involving carboxylation and carbonyl-exchange reactions.[2] It participates in the fixation, activation, and transfer of carbon dioxide by forming an *N*-carboxybiotin in which N1 was established as the point of attachment. The chemical reactivity of the

Table I. ^{15}N NMR Chemical Shifts and Coupling Constants for Biotin and Model Compounds

compd (concn, M)	solvent	δ^{15}N ($^1J_{NH}$, Hz) N1	N3
2-imidazolidinone (3) (1)	H_2O	294.0	294.0
(1.8)	H_2O	294.5	294.5
(1)	0.1 N $NaHCO_3$	294.3	294.3
(2)	$(CH_3)_2SO$	296.7 (93)	296.7 (93)
desthiobiotin (**2**) (saturated soln)	0.1 N $NaHCO_3$	276.8	282.5
(0.7)	$(CH_3)_2SO$	279.3 (90)	285.0 (91)
biotin (1) (saturated soln)	0.1 N $NaHCO_3$	283.6	292.6
(0.3)	$(CH_3)_2SO$	285.6 (94)	294.7 (92)

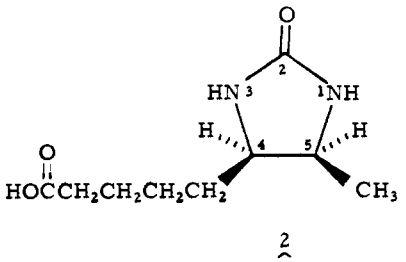


1

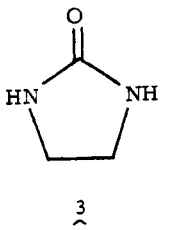
the two ureido nitrogens of **1** is strikingly different. Thus, N1- and N3-acylated products in an 100:7 ratio resulted from the reaction of methyl chloroformate with biotin methyl ester.[4a] The low proportion of the N3 product was attributed to steric hindrance to the approach of the reagent to N3 because of the presence of the carboxylbutyl side chain. A similar argument was employed[4b] to account for the difference in exchange rates for the ureido protons with ethanol.

Because of the lack of sensitive physical methods to distinguish between the two nitrogens of **1** in solution, we have examined the ^{15}N NMR spectra in the hope of providing a useful probe for determining how protein systems interact with biotin.

The ^{15}N chemical shifts[5] and one-bond NH coupling constants for biotin and two model compounds, desthiobiotin (**2**), and 2-imidazolidinone (**3**), are given in Table I. For both biotin and



2



3

desthiobiotin, two well-resolved ^{15}N resonances are observed in the proton-decoupled spectra. The two resonances in desthiobiotin

[†] Contribution No. 6345 from these laboratories.

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(5) The ^{15}N NMR spectra were recorded on a Bruker WH-180 Fourier-transform quadrature-detection spectrometer operating at 42 kG (18.25 MHz for ^{15}N and 180 MHz for ^{1}H) with 25-mm sample probe. Nitrogen chemical shifts are reported in ppm upfield of 1 M $H^{15}NO_3$.

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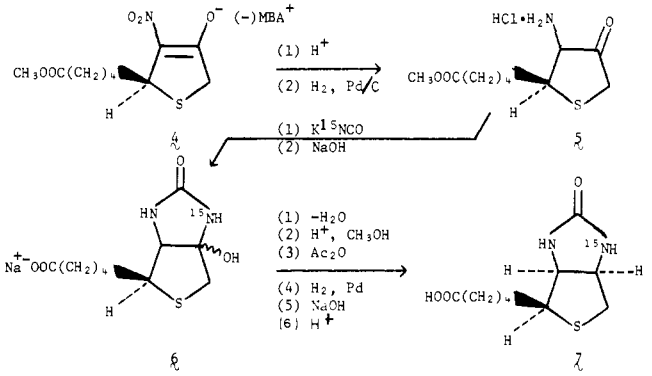
were assigned on the basis of selective decoupling. The coupled spectrum shows two broad doublets corresponding to one-bond NH splittings of 90 and 91 Hz. Selective irradiation of the protons of the methyl group at C5 changed the downfield ^{15}N doublet into a broad doublet of doublets. This behavior is consistent with the assignment of the downfield resonance to N1, with a three-bond coupling of the methyl protons (typical[6,7] $^3J_{NH}$ = 2–4 Hz) to N1 which can be removed by the selective decoupling, and also with the residual splitting due to the C4 proton.

The two resonances of biotin were assigned both by off-resonance decoupling and by synthesis of biotin specifically labeled with ^{15}N at N1. Explicit assigments of the biotin proton chemical shifts were necessary for the proton off-resonance decoupling experiments. The ureido proton resonances are observed at 220 MHz as two broad singlets at δ 6.45 and 6.37. On the basis of homonuclear decoupling experiments, the downfield ureido resonance was assigned to H3.

On irradiation with a coherent decoupling field (γH_2), the biotin one-bond NH couplings (J) are reduced and the long-range splittings effectively removed. The residual coupling (J'), which is linearly dependent on the difference ($\Delta\nu$) between the resonance frequency of the directly bonded proton and the decoupler frequency, goes to zero when the decoupler frequency coincides with the proton resonance.[8] Thus, $J' = J\Delta\nu/(\gamma H_2)$. As the proton decoupler was moved to higher field for biotin, the residual coupling of the nitrogen resonance centered at 294.7 ppm went to zero before the residual coupling of the resonance centered at 285.6 ppm. Therefore, the resonance centered at 294.7 ppm corresponds to the nitrogen directly coupled to the downfield unreido proton, H3. Thus, the chemical shift of N3 is 294.7 and of N1 is 285.6 ppm.

Synthesis of (+)-[1-^{15}N]biotin was achieved by the multistep procedure of Vasilevskis et al.,[9] starting with the (−)-α-methylbenzylamine salt of methyl 5-(2,5-dihydro-4-hydroxy-3-nitrothien-2-yl)pentanoate (**4**) kindly provided by Hoffmann-LaRoche, Inc., and using ^{15}N-labeled KNCO[10] with the reduced nitroketone to introduce the label unequivocally (**6**) into the eventual N1 position of (+)-biotin (**7**). The proton-decoupled spectrum of the N1-labeled biotin in 0.1 N $NaHCO_3$ gave a single resonance at 283.5 ppm (see Figure 1). This result confirms the off-resonance decoupling assignment.



The nitrogen chemical shift range of ureas[11] is between 270 and 320 ppm. The equilibrium concentration of the imino tautomer is usually small. Comparison of the chemical shifts of *O*-methylisourea[12] and tetramethylurea indicates that the imine nitrogen should be downfield from urea nitrogens by about 80 ppm. The range of nitrogen chemical shifts observed for **1–3** is consistent with all of these nitrogens being of the urea type. Furthermore, the differences in nitrogen shifts among the homologous ureas **1–3** are consistent with known β-, γ-, and δ-substituent effects on ureas.[13,14] Downfield shifts in the range 12–19 ppm result from replacing a hydrogen on the α carbon by a methyl group in acrylic ureas.[13] Similar β-substitutent effects can account for most of the shift differences observed between 2-imidazolidinone (**3**) and desthiobiotin (**2**).

γ-Substituent effects are sensitive to molecular conformation and increase with steric crowding at the nitrogen induced by the γ substituent. In acyclic ureas, upfield shifts range from 2 to 4 ppm.[13] For cyclic compounds, where the relationship between the nitrogen and γ carbon is fixed, gauche effects are pronounced. Koch and co-workers[14] reported that the substituent effects for sterically hindered urea nitrogens are increased by 10–12 ppm (upfield) for γ substituents and 4 ppm (downfield) for δ substituents.[15]

The 5.7-ppm difference between the desthiobiotin resonances falls within the range expected for γ and δ effects on N3 arising from the carboxybutyl side chain at C4. For biotin molecules in

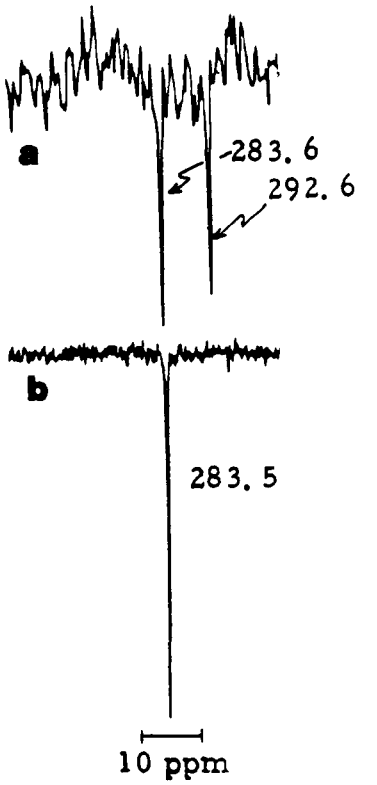


Figure 1. (a) Natural-abundance ^{15}N NMR spectrum of (+)-biotin in 0.1 N $NaHCO_3$. (b) ^{15}N NMR spectrum of (+)-biotin, specifically labeled with ^{15}N (93%) at N1, in 0.1 N $NaHCO_3$.

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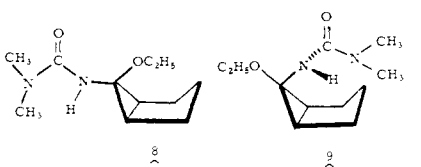
(15) For example, the NH shift in **9** is 10.6 ppm upfield from the corresponding shift in **8**.[14]



the crystal,[16] the sulfur is in the endo position forming 84 and 86° dihedral angles with N3 and N1 which constrains Cl′ to a pseudoequatorial position and a 43° dihedral angle to N3. The solution conformation is similar as indicated by the proton-coupling constants.[17,18] The difference between the interactions of N1 and N3 with Cl′, combined with the presence of a γ-sulfur atom, could reasonably cause the N3 nitrogen shift of biotin to be only 2 ppm downfield from that of 2-imidazolidinone (**3**) and yet 9 ppm upfield from that of N1.

The steric hindrance which causes these ^{15}N-shift differences is in accord with the observed retardation of chemical reactions at N3 relative to N1.[2,4] The availability of (+)-biotin unequivocally labeled at N1 suggests its use as a mechanistic probe for following the biological carboxylation and transcarboxylation of biotin and investigating the interaction between biotin and avidin, inter alia.

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Rearrangement of Vobasine to Ervatamine-Type Alkaloids Catalyzed by Liver Microsomes

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It has been shown[1] that the in vitro transformation of the alkaloids of the vobasine series into those of the ervatamine series may be readily accomplished in three steps (Figure 1): oxidation of the starting alkaloid to the corresponding *N*-oxide, rearrangement of this *N*-oxide induced by its treatment with trifluoroacetic anhydride ("modified Polonovski reaction "[2]), and reduction of the intermediate iminium ion.

In this way dregamine and tabernaemontanine have been transformed stereospecifically and in high yields to 20-*epi*-ervatamine and ervatamine, respectively. This sequence of reactions is in good agreement with the proposal that the biosynthesis of the ervatamine alkaloids could involve the intermediacy of vobasine-type systems which contain the ethanamine unit of tryptophan.

In the context of this hypothesis, it was interesting to examine whether an enzyme preparation would also be able to catalyze the transformation of vobasine into ervatamine-type alkaloids. Cytochrome P-450 dependent monooxygenases were chosen since they are known to be able to catalyze both the oxidation of tertiary amines[3] by dioxygen and NADPH (step a, Figure 1) and the reduction of various substrates including azo[4] and nitro compounds,[5] *N*-oxides,[6] and nitroxides[7] by NADPH (reactions analogous to step c of Figure 1).

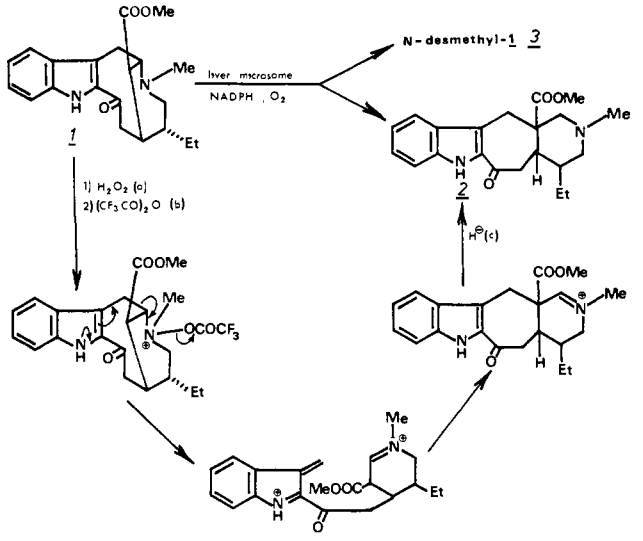

Figure 1.

Table I. Influence of Various Factors on the Formation of Metabolites **2** and **3**

conditions	formation rates of (in nmol per nmol of cytochrome P-450 per 30 min)[13] **2**[c]	**3**[c]
complete incubation system[a]	2	70
$-O_2$ (under argon)	0.4	4
−NADPH	<0.1	2
+ellipticine	0.6	20
after protein denaturation[b]	0.2	3

[a] 5 mM **1** + 1 mM NADPH + aerated microsomes (10^{-5} μM cytochrome P-450). [b] With microsomes boiled 5 min at 95 °C. [c] Mean values calculated from 2 to 5 experiments; the errors limits vary from ±20% to ±90% for very low values.

Moreover, catalysis of step b by the iron of cytochrome P-450 appears likely since it has been reported that iron salts[8] catalyze some reactions of *N*-oxides that can be also equally performed by using trifluoroacetic anhydride with the conditions of the modified Polonovski reaction.[2] The present paper describes the results obtained by incubation of dregamine (**1**) with rat liver microsomes in the presence of NADPH and O_2, which leads to 20-*epi*-vervatamine (**2**), and shows that this reaction is catalyzed by microsomal cytochrome P-450 dependent monooxygenases.

Dregamine hydrochloride[9] (5 mM) was incubated at 37 °C with a suspension of liver microsomes[10] from phenobarbital[11] pretreated rats in phosphate buffer at pH 7-4 (5 mg protein per mL; 2.1 nmol of cytochrome P-450 per mg protein) in the presence of

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