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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(16) De Titta, G. T.; Edmonds, J. W.; Stallings, W.; Donohue, J., *J. Am. Chem. Soc.* **1976**, *98*, 1920-1926.
(17) Lett, R.; Marquet, A. *Tetrahedron* **1974**, *30*, 3365-3377 and 3379-3392.
(18) (a) Griesser, R.; McCormick, D. B. *Arch. Biochem. Biophys.* **1974**, *160*, 667-8. (b) Sigel, H.; McCormick, D. B.; Griesser, R.; Prijis, B.; Wright, L. D. *Biochemistry* **1969**, *8*, 2687-2695.

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).

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

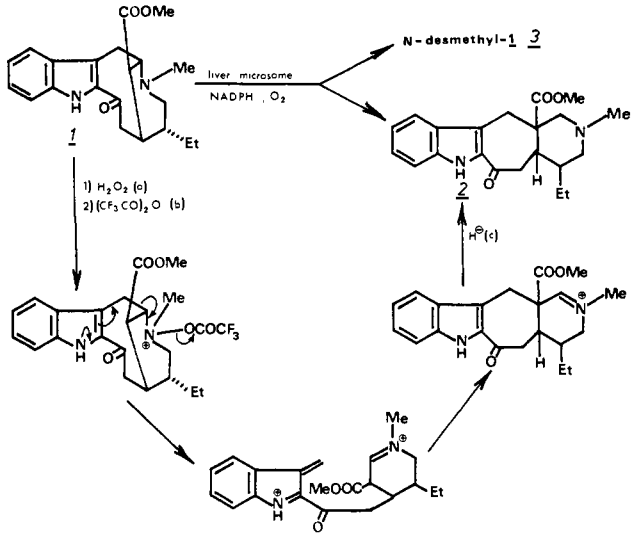

Figure 1.

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.

(1) Husson, A.; Langlois, Y.; Riche, C.; Husson, H.-P.; Potier, P. *Tetrahedron* **1973**, *29*, 3095.
(2) (a) Cavé, Ad.; Kan-Fan, C.; Potier, P.; Le Men, J. *Tetrahedron* **1967**, *23*, 4681. (b) Ahond, A.; Cavé, Ad.; Kan-Fan, C.; Husson, H.-P.; de Rostolan, J.; Potier, P. *J. Am. Chem. Soc.* **1968**, *90*, 5622. (c) Husson, H.-P.; Chevolot, L.; Langlois, Y.; Thal C.; Potier, P. *J. Chem. Soc., Chem. Commun.* **1972**, 930. (d) Potier, P. *Rev. Latino-Am. Quim.* **1978**, *9*, 47. Potier, P. In "Indole and Biogenetically Related Alkaloids"; Phillipson, J. D., Zenk, M. H., Eds.; Academic Press: London, 1980, p 159.
(3) (a) Gorrod, J. W. *Chem. Biol. Interact.* **1973**, *7*, 289. (b) Wehleke, H. *Drug Metab. Dispos.* **1973**, *1*, 299. (c) Beckett, A. H.; Morton, D. M. *J. Pharm. Pharmacol. Suppl.* **1966**, *18*, 88-91. (d) B. Wilson and S. Orrenius, *Chem. Biol. Interact.* **1971**, *3*, 313.
(4) Fujita, S.; Peisach, J. *J. Biol. Chem.* **1978**, *253*, 4512.
(5) Gillette, J. R.; Kamm, J. J.; Sasame, H. A. *Mol. Pharmacol.* **1968**, *4*, 541.
(6) Sugiura, M.; Iwasaki, K.; Kato, R. *Mol. Pharmacol.* **1976**, *12*, 322.
(7) Rosen, G. M.; Rauckman, E. J. *Biochem. Pharmacol.* **1977**, *26*, 675.
(8) Scherer, C. A.; Dorschel, C. A.; Cook, J. M.; Lequesne, P. W. *J. Org. Chem.* **1972**, *37*, 1083.
(9) We are grateful to A. Husson, M. Colin, and A.-M. Bui for supplying alkaloids **1** and **2**.
(10) Prepared by a classical procedure: Frommer, U.; Ullrich, V.; Staudinger, H. J. *Z. Physiol. Chem.* **1970**, *351*, 903.
(11) Used as a good inducer of cytochromes P_{450}.[10]

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NADPH. After protein precipitation by the addition of acetone and extraction of the aqueous phase at pH 8 with ethyl acetate, the reaction mixture was separated by thin-layer chromatography (SiO_2, 99.75:0.25 AcOEt–NH_4OH) and each fraction analyzed by HPLC (μ-Porasil column, 99.75:0.25:0.02 CH_2Cl_2–CH_3OH–$(CH_3)_3N$).

Formation of 20-*epi*-ervatamine under these conditions was unambiguously shown by comparison of the TLC and HPLC retention time and mass and UV spectral data for the metabolite purified with those of an authentic sample.[9] The major metabolite[12] of dregamine is its N-demethylation product **3**, the structure of which has been shown by the mass spectrum of a sample purified by HPLC ($M^{+\cdot}$ 254) and its transformation back to dregamine (**1**) upon Eschweiler–Clark reductive methylation.[13]

About 4 and 140 nmol of **2** and **3** are, respectively, formed per mg of protein and per 30 min in the aforementioned conditions.

Table I shows the influence of various factors on the yields of formation of metabolites **2** and **3**. The yields were dramatically reduced upon heat denaturation of the protein before incubation, showing that formation of **2** and **3** is almost totally enzyme dependent. These yields were very low in the absence of NADPH or O_2, the necessary cofactors of cytochrome P-450 dependent microsomal monooxygenases,[14] and were greatly decreased in the presence of ellipticine (1 mM), known to be an efficient inhibitor of these monooxygenases[15] (Table I).

Furthermore, dregamine (**1**) readily binds to the hydrophobic active site of microsomal cytochrome P-450 as shown by visible spectroscopy. The difference spectrum produced by progressive addition of dregamine to a rat liver microsomal suspension exhibits a peak around 395 nm and a trough at 420 nm. It corresponds to the transition of originally low-spin cytochrome P-450 iron(III) to the high-spin state upon binding of **1** to a protein active site near the heme and is characteristic of the formation of a cytochrome P-450 substrate complex.[14] The binding affinity of **1** for cytochrome P-450 from liver microsomes of phenobarbital pretreated rats is relatively high since the concentration of **1** producing the half-maximum formation of the difference spectrum is about 10^{-5} M.

Taken together, these results are in agreement with the involvement of cytochrome P-450 in the microsomal oxidative demethylation of dregamine to the secondary amine **3** and its isomerization to 20-*epi*-ervatamine (**2**).

Oxidations of tertiary amines resulting in *N*-oxide formation or N-dealkylation are well-known microsomal reactions;[3] radical cations[16] of the starting amines ($RCH_2NR'R''^{+\cdot}$) iminium ions ($RCH{=}NR'R''^{+}$) and carbinolamines ($RCHOHNR'R''$) have been proposed as intermediates in these N-dealkylation reactions.[17]

It seems possible that 20-*epi*-ervatamine was derived either from a rearrangement of the *N*-oxide of **1** catalyzed by the iron cytochrome P-450 (iron(III) playing the same role as $COCF_3$ in the modified Polonovski reaction) or from a free-radical rearrangement of the radical cation formed by one-electron oxidation of **1**.

Whatever the exact mechanism may be, the present results describe an enzymatic equivalent of the modified Polonovski reaction for the conversion of vobasine to ervatamine-type alkaloids, providing a further argument in favor of the previously proposed biogenetic filiation between alkaloids of the vobasine and ervatamine types. It also supports the hypothesis of the modified Polonovski reaction being "biomimetic".

(12) HPLC analysis reveals the presence of many other products in the reaction mixture, either derived from **1** or from low-molecular weight compounds from microsomes. This made quantitative analysis difficult and explains the relatively high errors on some determinations (Table I).

(13) Gorman, M.; Sweeney, J. *Tetrahedron Lett.* **1964**, 3105.

(14) Ullrich, V. *Angew. Chem., Int. Ed. Engl.* **1972**, *11*, 701.

(15) (a) Lesca, P.; Lecointe, P.; Paoletti, C.; Mansuy, D. *Biochem. Pharmacol.* **1978**, *27*, 1203. (b) Lesca, P.; Rafidinarino, E.; Leconite, P.; Mansuy, D. *Chem. Biol. Interact.* **1979**, *24*, 189.

(16) Ashley, B. L.; Griffin, B. W. *Mol. Pharmacol.* **1981**, *19*, 146.

(17) (a) Nguyen, T. L.; Gruenke, L. D.; Castagnoli, N. *J. Med. Chem.* **1976**, *19*, 1168. (b) Gorrod, J. W.; Genner, T. *Essays Toxicol.* **1975**, *6*, 35. (c) Gescher, A.; Huckman, J. A.; Stevens, M. F. G. *Biochem. Pharmacol.* **1979**, *28*, 3235.

Effect of Molecular Structure on Mesomorphism. 12.[1] Flexible-Center Siamese-Twin Liquid Crystalline Diesters—A "Prepolymer" Model

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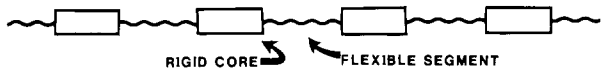
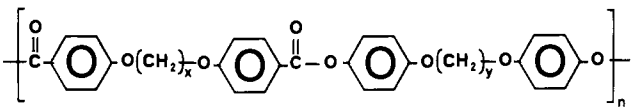
The vast majority of mesogenic (liquid crystalline) molecules are cigar-shaped structures having a rigid central core. This core is often composed of aromatic rings (or conformationally locked saturated rings) joined through a linking group such as —COO—, —CH=N— or —N=NO—.[2] In fact, the structural requirement of a rigid core is often given as necessary for nematic behavior in small molecule liquid crystals.[3,4] There have been recent reports of polymeric liquid crystals in which a small molecule liquid crystal moiety has been incorporated into the polymer backbone.[5,6] This is schematically shown below. An example of such a polymer



is compound **1**.[5d] All 30 polymers exhibited nematic phases on



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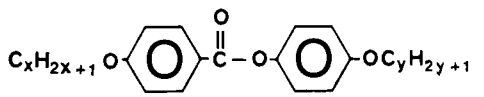
heating. Using calorimetrically determined (DSC) binary phase diagrams, we have established the nematic nature of the polymeric mesophase.[7] This phase assignment is supported by X-ray diffraction photographs of this mesophase in which only a single, diffuse ring at 4.4 Å was observed.[8] From viscosity data, the polymers **1** have estimated average molecular weights, M_w, ranging from 16 200 to 19 200.[8] These polymers (**1**) are structurally related to the mesogenic small molecule family of 4-alkoxyphenyl 4′-alkoxybenzoates (**2**) which have a rigid central core with flexible tail groups.



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(1) For part 11 in this series, see: Griffin, A. C.; Buckley, N. W.; Hughes, W. E.; Wertz, D. L. *Mol. Cryst. Liq. Cryst. Lett.* **1980**, *64*, 139.

(2) A comprehensive listing of all liquid crystalline structures known up to 1974 is given in: Demus, D.; Demus, H.; Zaschke, H. "Flussige Kristalle in Tabellen" VEB Deutscher Verlag fur Grundstoffindustrie: Leipzig, 1974.

(3) Gray, G. W. In "The Molecular Physics of Liquid Crystals"; Luckhurst, G. R., Gray, G. W., Eds.; Academic Press: London, 1979; Chapter 1 and references cited therein.

(4) Destrade, C.; Tinh, N. H.; Gasparoux, H. *Mol. Cryst. Liq. Cryst.* **1980**, *59*, 273.

(5) (a) Roviello, A.; Sirigue, A. *J. Polym. Sci, Polym. Lett. Ed.* **1975**, *13*, 455. (b) Guillon, D.; Skoulios, A. *Mol. Cryst, Liquid Cryst. Lett.* **1978**, *49*, 119. (c) Blumstein, A.; Sivaramakrishnan, K. N.; Clough, S. B.; Blumstein, R. B. *Ibid.* **1979**, *49*, 255. (d) Griffin, A. C.; Havens, S. J.; *Ibid.* **1979**, *49*, 239. (e) Strzelecki, L.; van Luyen, D. *Eur. Polym. J.* **1980**, *16*, 299. (f) van Luyen, D.; Strzelecki, L. *Ibid.* **1980**, *16*, 303. (g) van Luyen, D.; Liebert, L.; Strzelecki, L.; *Ibid.* **1980**, *16*, 307.

(6) For recent reviews, see the following: (a) Amerik, Y. B. *Itogi Nauki Tekh.: Tekhnd. Vysokomol. Soedin.* **1978**, *12*, 177. (b) Blumstein, A., *Contemp. Top. Polym. Sci.*, *3*, 79 (1979). (c) Blumstein, A. *Adv. Chem. Ser.* **1978**, *No. 74*. (d) Samulski, E. T.; Du Pre, D. B. *Adv. Liq. Cryst.* **1979**, *4*, 121. (e) Blumstein, A. *Polym. News* **1979**, *5*, 254.

(7) Griffin, A. C.; Havens, S. J. *J. Polym. Sci., Polym. Lett. Eds.* **1980**, *18*, 259.

(8) Griffin, A. C.; Havens, S. J. *J. Polym. Sci., Polym. Phys.* **1981**, *19*, 951.

0002-7863/81/1503-4957$01.25/0