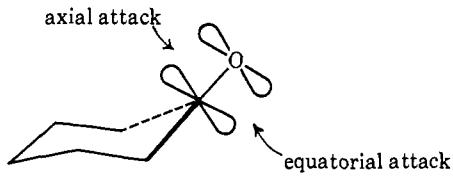
stants for complex formation are also given in the table.

Attention is first drawn to cyclohexanones substituted in the three, four, and five positions. We see from **1–3** that additions of methyl groups equatorially in the three and five positions, or an equatorial *t*-butyl group in the four position has little effect on k_c. These observations are consonant with the idea that equatorial substitution at these carbons will not hinder approach to the nucleophilic π system of the carbonyl n,π^* excited singlet, and that the excited state is related to the chair conformation of cyclohexanone. On the other hand, comparison of the k_c values for **3** and **4** demonstrates the adverse effect on the rate of complex formation of an axial methyl substitution at the three or five position. The addition of a second axial methyl group (**5**) has no additional effect on k_c. Axial substituents in the three and five positions should interfere with access to the carbonyl from the same side as the substituents, thus making *axial attack* less favorable while having no effect on *equatorial attack* (see structure I). The constancy



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of k_c in going from **4** to **5** then implies that one axial methyl group in the three or five position is sufficient to block nearly all the axial attack.

Looking next at the two- and six-substituted cyclohexanones (**6–10**) we see from a comparison of k_c values for **1**, **6**, and **7** that the introduction of equatorial methyl groups in these positions causes a significant decrease in the rate constant for complex formation. Models suggest that this decrease is primarily due to steric inhibition of axial attack on the ketone excited singlet. The enhancement of the rate constant for complex formation with the *substitution* of axial methyls for equatorial methyls in the two or six position (see **7**, **8**, and **10**) lends further support to this hypothesis. In addition, however, a comparison of the k_c values for **6**, **8**, and **10** suggests that the *introduction* of axial substituents at these carbons actually leads to an increase in the overall rate constant for complex formation. This may mean that the introduction of an axial methyl group in the two or six position results in a subtle change in the electronic distribution in the n,π^* excited state causing an increase in the nucleophilicity of the ketone singlet and a faster rate of complex formation.[5]

The sensitivity of k_c to steric factors is consistent with "steric approach control" of the rate of attack on the nucleophilic π system of the cyclohexanone n,π^* excited singlet. Similar steric effects have been observed on the rates of reduction of substituted cyclohexanones by nucleophilic metal hydrides,[6] a ground-state process now thought to be governed by "steric approach control."

Work is currently in progress to determine whether the oxetanes formed upon photolysis of the cyclohexanones with *t*-DCE are those resulting from axial or equatorial attack.

(5) Cyclohexanones substituted with axial methyl groups in the two or six position have both longer fluorescence lifetimes (τ_s) and larger extinction coefficients ($\epsilon_{\lambda_{max}}$) than the analogous cyclohexanones with equatorial methyls. Also in cyclohexyl radicals, significant interaction between hydrogens in the two and six positions and the radical center is observed only for axial hydrogens. See M. C. R. Symons, *Nature*, **198**, 1196 (1963).

(6) See, for example, J. Klein, E. Dunkelblum, E. L. Eliel, and Y. Senda, *Tetrahedron Lett.*, 6127 (1968).

(7) National Institutes of Health Predoctoral Fellow, 1966–1970.

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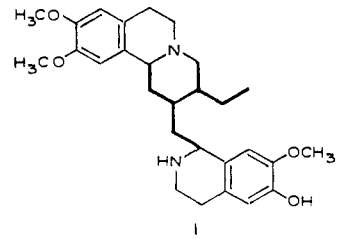
Studies on Indole Alkaloid Biosynthesis. V.[1] The Role of Glycine

Sir:

It has been established that the labeled carbon of glycine-2-^{14}C can be utilized by plants as a "C_1" source. For example, *Nicotiana rustica* L. incorporates the α carbon of glycine into the N-methyl group of nicotine as efficiently as the methyl group of methionine or choline, and ten times as rapidly as formate.[2] It has also been shown that glycine-1-^{14}C is utilized in the formation of the fungal metabolite, echinulin.[3] More recently Gear and Garg reported that glycine-2-^{14}C is a specific precursor of the $C_{9\text{-}10}$ unit in cephaeline (I,



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darkened lines), while glycine-1-^{14}C is a very poor precursor.[4] It was this latter publication which was of particular interest to us since the $C_{9\text{-}10}$ unit present in cephaeline is identical with the unrearranged nontryptophan moiety (darkened lines in II) in the Corynanthe alkaloids, for which mevalonoid origin has been established.[5] The question of glycine involvement in the biosynthesis of these indole alkaloids was therefore undertaken.

Glycine-1-^{14}C and -2-^{14}C of the same specific activity were fed to mature *Vinca rosea* L. plants by means of the cotton wick method over a period of 9 days. Isolation of the alkaloid ajmalicine (II) revealed no incorporation with glycine-1-^{14}C but reasonably good incorporation with glycine-2-^{14}C.

In order to determine the location of label in the alkaloid derived from the glycine-2-^{14}C experiment, a systematic degradation of the active ajmalicine was performed as outlined in Scheme I. The incorpora-

(1) Part IV: J. P. Kutney, V. R. Nelson, and D. C. Wigfield, *J. Amer. Chem. Soc.*, **91**, 4279 (1969).

(2) R. U. Byerrum, R. L. Hamill, and C. D. Ball, *J. Biol. Chem.*, **210**, 645 (1954).

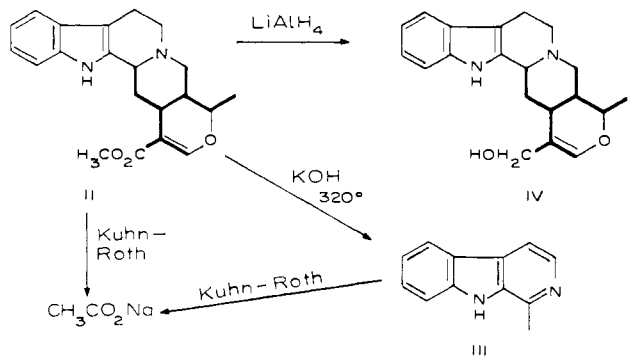
(3) A. J. Birch, G. E. Blance, S. David, and H. Smith, *J. Chem. Soc.*, 3128 (1961).

(4) J. R. Gear and A. K. Garg, *Tetrahedron Lett.*, 141 (1968).

(5) (a) For a general review and a collection of references see A. R. Battersby, *Pure Appl. Chem.*, **14**, 117 (1967); (b) for more recent references, see A. R. Battersby, A. R. Burnett, and P. G. Parsons, *J. Chem. Soc., C*, 1187 (1969).

tions and degradations were carried out several times by different research workers so as to confirm the reproducibility of the results.

Scheme I



A summary of the results of the various experiments is given in Table I.

Table I. Results of Incorporation of Glycine into Ajmalicine (II)

Expt	Compd fed	% incorporation into ajmalicine	% of alkaloid specific activity in degradation products[a] CH_3CO_2Na	Harman (III)	Ajmalicinol (IV)
1	Glycine-1-^{14}C	0.0008			
2	Glycine-2-^{14}C	0.26	0.41		
3	Glycine-2-^{14}C	0.17	0.86	69	
4	Glycine-2-^{14}C	0.48	0.58	59	63
5	Glycine-2-^{14}C	0.31	0.78[b]	78	79

[a] The specific activity in ajmalicine is taken as 100%. [b] This value refers to sodium acetate obtained in Kuhn–Roth oxidation of harman. The values in experiments 2, 3, and 4 refer to oxidation on ajmalicine.

An analysis of the results quickly reveals that high levels of activity reside in two portions of the alkaloid: (1) the tryptophan unit containing 60–80%; (2) the methyl group of the ester containing approximately 20–35%. The C_{10} unit contains very little activity (approximately 1–4%).

Plausible explanations for the obtained results can be advanced. For example, it has been shown conclusively[6] that in microorganisms tryptophan is a product of the shikimate–chorismate pathway and that the final biosynthetic step, catalyzed by tryptophan synthetase, is the replacement of the glycerol phosphate moiety of indolyl-3-glycerol 3′-phosphate by serine to form the side chain of this amino acid. This same tryptophan synthetase activity has been demonstrated in plants.[7] Furthermore, the activity of the enzyme, serine aldolase, responsible in mammalian systems for the interconversion of glycine and serine has also been shown in plant systems.[8] Since both glycine and serine have very recently been shown to be present in *Vinca* plants,[9] it is attractive to postulate that glycine can be utilized in the biosynthesis of the tryptophan unit in *V. rosea*. The high level of activity found in the degradation product, harman (III), is explicable in these terms.

The presence of significant activity in the methyl group of the ester function is merely an indication that in *V. rosea*, degradation to a "C_1" can occur, a process observed previously.[2]

Of particular interest is the finding that very little activity is found in the C_{10} unit, in contrast to the results obtained by Gear and Garg[4] in their experiments with *Cephaelis ipecacuanha* plants. Our results suggest that in *V. rosea*, glycine-2-^{14}C is not a specific precursor of the C_{10} unit.

It is relevant at this point to note the recent studies by Shah and Rogers[10] on terpenoid biosynthesis in green plants. They suggest that acetyl CoA, an established intermediate, may be formed from carbon dioxide *via* the route, carbon dioxide → glycollate → glyoxylate → glycine → serine → pyruvate → acetyl CoA. The obvious implication of glycine involvement in acetyl CoA and, thereby in turn, in biosynthesis of the C_{10} unit required in ajmalicine does not receive strong support from our results. Whether the postulated involvement of glycine in the cephaeline biosynthesis[4] reveals a different biosynthetic pathway in that plant system relative to *V. rosea* remains an open question.

Finally, the nonincorporation of glycine-1-^{14}C into ajmalicine is readily understood. The conversion of glycine to both a "C_1" unit and tryptamine (*via* serine) entails the loss of the carboxyl group.[2, 10]

Further experiments to provide additional information relevant to the above are now in progress.[11]

Acknowledgment. Financial aid from the National Research Council of Canada is gratefully acknowledged.

(6) (a) J. R. Mattoon, "Biogenesis of Natural Compounds," 2nd ed, P. Bernfeld, Ed., Pergamon Press, New York, N. Y., 1967, p 34; (b) I. D. Spenser, *Compr. Biochem.*, 20, 330 (1968).

(7) L. Fowden, "Plant Biochemistry," J. Bonner and J. E. Varner, Ed., Academic Press, New York, N. Y., 1965, p 381.

(8) Reference 7, p 379.

(9) R. R. Paris and R. L. Girre, *C. R. Acad. Sci. Paris, D*, 268, 62 (1969).

(10) S. P. J. Shah and L. J. Rogers, *Biochem. J.*, 114, 395 (1969).

(11) After this communication was submitted for publication, two communications have appeared: (a) A. K. Garg and J. R. Gear, *Tetrahedron Lett.*, 4377 (1969); (b) A. K. Garg and J. R. Gear, *Chem. Commun.*, 1447 (1969). In both instances, the specific incorporation of glycine into the $C_{9\text{-}10}$ unit is reported. These authors suggest that "glycine may be a fundamental precursor of the $C_{9\text{-}10}$ unit in alkaloids." Our results are not in agreement with that statement.

(12) To whom inquiries should be sent.

(13) Visiting professor, summer 1969.

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Thallium in Organic Synthesis. XIV. Orientation Control in an Electrophilic Aromatic Substitution Reaction[1,2]

Sir:

We describe in this paper control over *ortho*, *meta*, or *para* substitution in the same electrophilic aromatic substitution reaction (thallation), and the application

(1) We gratefully acknowledge financial support of this work by the Smith Kline & French Laboratories, Philadelphia, Pa. 19101.

(2) Part XIII: A. McKillop, B. P. Swann, M. J. Zelesko, and E. C. Taylor, *Angew. Chem.*, in press.