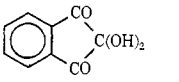
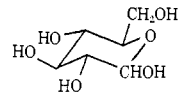
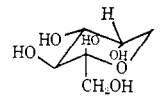
the hydroxyl protons of water and simple alcohols, as shown by the preference of the former species for deuterium over protium in isotopic exchange equilibria with the latter (Table I). This tends to confirm recent theo-

Table I. Isotopic Fractionation Factors for *gem*-Diols, Hemiacetals, and Alcohols[a]

No.	Compound	ϕ
1	Chloral hydrate, $Cl_3CCH(OH)_2$	1.23 ± 0.08
2	Ninhydrin, 	1.24 ± 0.20
3	D-Glucose, 	1.28 ± 0.17
4	D-Fructose,[b] 	1.23 ± 0.02
5	Methanol, CH_3OH	0.96 ± 0.05
6	Ethanol, CH_3CH_2OH	1.05 ± 0.14
7	2-Propanol, $(CH_3)_2CHOH$	1.07 ± 0.30
8	2-Methyl-2-propanol, $(CH_3)_3COH$	0.97 ± 0.15

[a] Determined by the method of ref 3 and 4 using the dependence of chemical shift of the solvent proton in protium oxide and 95% deuterium oxide on the mole fraction of solute. [b] D-Fructose in aqueous solution is 32% in the furanose form and 68% in the pyranose form. B. Andersen and H. Degn, *Acta Chem. Scand.*, **16**, 215 (1962).

retical conclusions[1] that there are large barriers to rotation about the C–O bonds in such compounds.

Table I shows ϕ (isotopic fractionation factors) for the relevant compounds. These quantities are equilibrium constants for the isotopic exchange reaction of eq 1.

$$SOH + HOD \rightleftharpoons SOD + HOH \quad (1)$$

As is well known from both the theory and practice of isotope effects,[2] deuterium will accumulate in preference to protium during an exchange reaction in those sites where the overall binding to hydrogen is tighter (*i.e.*, where its force constants are larger). Thus if the average binding to hydrogen in the species SOH (D) is looser than in bulk water, $\phi \leq 1$ for eq 1, while if binding in SOH (D) is tighter than in bulk water, $\phi \geq 1$. The data of Table I show that simple aliphatic alcohols (compounds 5–8) have hydroxyl groups in which the average binding of the hydrogen is similar to the binding in water (ϕ averages 1.01 with about 15% error for compounds 5–8), while the *gem*-diols (compounds 1 and 2) and hemiacetals (compounds 3 and 4) have substantially tighter binding of their hydroxyl hydrogens (ϕ averages 1.25 with about 15% error for compounds 1–4).

The isotopic fractionation factors were determined by the Kresge–Allred[3]–Gold[4] technique using nmr chemical shifts. Although crude, this measure of binding is unambiguous and probably is the best way to demonstrate experimentally the conclusion of Jeffrey, Pople and Radom.[1]

(1) G. A. Jeffrey, J. A. Pople, and L. Radom, *Carbohyd. Res.*, **25**, 117 (1972).
(2) E. K. Thornton and E. R. Thornton, "Isotope Effects in Chemical Reactions," C. J. Collins and N. S. Bowman, Ed., Van Nostrand Reinhold Co., New York, N. Y., 1970, Chapter 4. See also S. R. Hartshorn and V. J. Shiner, Jr., *J. Amer. Chem. Soc.*, **94**, 9002 (1972).
(3) A. J. Kresge and A. L. Allred, *J. Amer. Chem. Soc.*, **85**, 1541 (1963).
(4) V. Gold, *Proc. Chem. Soc., London*, 141 (1963).
(5) On leave from the University of Costa Rica, San José, Costa Rica.
(6) National Science Foundation Undergraduate Research Participant.
(7) Support of this research by the National Science Foundation and the National Institutes of Health is gratefully acknowledged.

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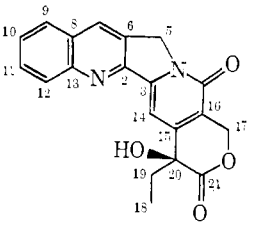
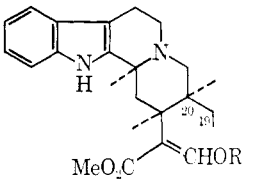
Biosynthesis of Camptothecin. I. Definition of the Overall Pathway Assisted by Carbon-13 Nuclear Magnetic Resonance Analysis[1]

Sir:

Camptothecin (**1**)[2] has been the subject of numerous synthetical and biochemical investigations due to early reports of its potent antitumor activity.[3] Biosynthetically, **1** also is unique, the first reported example of an alkaloid containing the pyrrolo[3,4-*b*]quinoline unit. Wenkert, *et al.*,[4a] suggested in 1967 that **1** might be formed *in vivo* from an indole alkaloid of the corynantheidine type (**2a**); more recently Winterfeldt[4b] has suggested a biosynthetic relationship between **1** and geissoschizine (**2b**). We considered an alternative



1

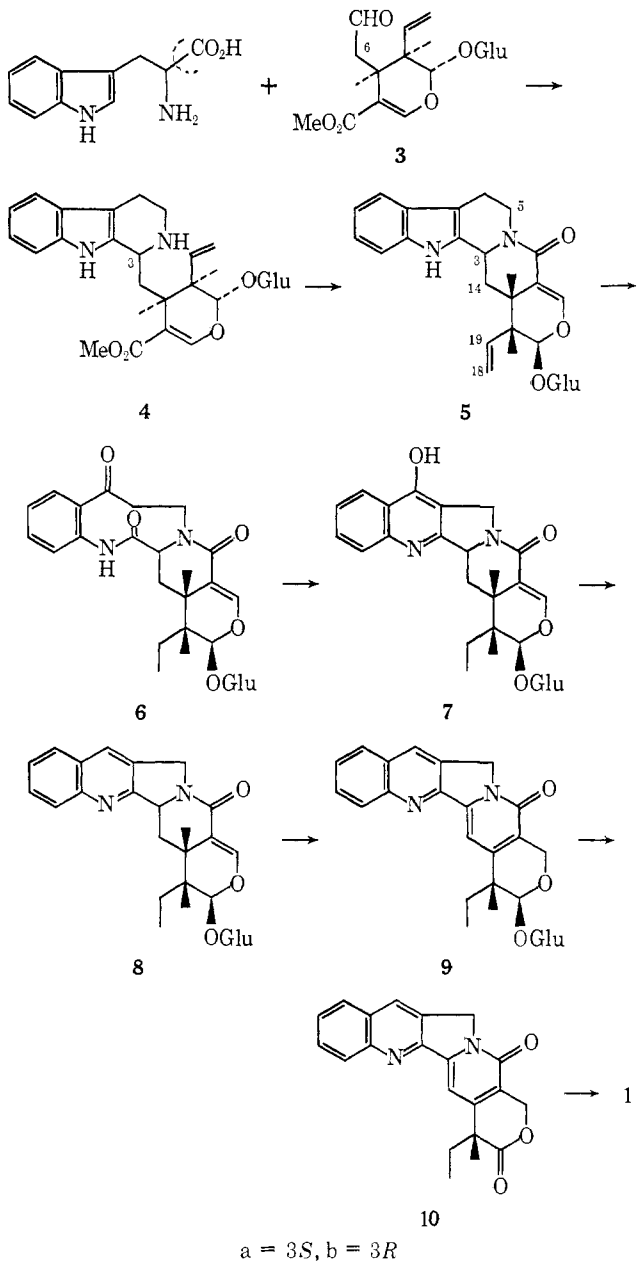


2a, R = CH_3
b, R = H; 19,20-dehydro

possibility for the biosynthesis of **1**, which arose out of the now detailed understanding of the biosynthesis of the indole alkaloids of *Catharanthus roseus* G. Don.[5–8] As outlined in Scheme I, the epimeric lactams (**5**), which are formed from isovincoside (strictosidine), **4a**,[9,10] and vincoside (**4b**),[9] respectively, could give rise *in vivo* to desoxycamptothecin (**10**) by a straightforward sequence of chemically sensible transformations.[11] We

(1) (a) Presented at the Fifth Natural Products Symposium, University of West Indies, Mona, Jamaica, Jan 7, 1974. (b) Supported in part by the National Institutes of Health (CA 13616).
(2) Numbered according to M. Shamma, *Experientia*, **24**, 107 (1968).
(3) A. G. Schultz, *Chem. Rev.*, **73**, 385 (1973).
(4) (a) E. Wenkert, K. G. Dave, R. G. Lewis, and P. W. Sprague, *J. Amer. Chem. Soc.*, **89**, 6741 (1967); (b) E. Winterfeldt, *Justus Liebigs Ann. Chem.*, **745**, 23 (1971).
(5) S. Escher, P. Loew, and D. Arigoni, *Chem. Commun.*, 823 (1970), and references cited therein.
(6) A. R. Battersby, *Accounts Chem. Res.*, **5**, 148 (1972).
(7) J. P. Kutney, J. F. Beck, C. Ehret, G. Poulton, R. S. Sood, and N. D. Westcott, *Bioorg. Chem.*, **1**, 194 (1971).
(8) (a) A. I. Scott, *Accounts Chem. Res.*, **3**, 151 (1970); (b) A. I. Scott, P. Reichardt, M. B. Slaytor, and J. G. Sweeney, *Bioorg. Chem.*, **1**, 157 (1971).
(9) A. R. Battersby, A. R. Burnett, and P. G. Parsons, *J. Chem. Soc. C*, 1193 (1969).
(10) K. T. D. DeSilva, G. N. Smith, and K. E. H. Warren, *Chem. Commun.*, 905 (1971).
(11) We do not mean to suggest that the biosynthesis of **1** proceeds by exactly this sequence of transformations. A similar hypothesis has been described by G. A. Cordell, *Lloydia*, **37**, 219 (1974).

Scheme I. Lactam Hypothesis for the Biogenesis of Camptothecin



a = 3*S*, b = 3*R*

now report experimental results from the feeding of labeled precursors to *Camptotheca acuminata* Decne. (Nyssaceae) plants that are in excellent support of our lactam hypothesis for the biosynthesis of **1**.

Preliminary feeding experiments were carried out in the summer and early fall (1971–1972) using intact 1-year old plants, cut shoots, and seedlings. Successful incorporation of established indole alkaloid precursors,[5–8] and the C-3 epimeric *mixture* of [5-$^3H_2$]- *or* [14-$^3H_2$]-**4**[12] was observed (Table I), but in no instance was this incorporation confirmed by degradation of the resulting radioactive **1**. While this work was in progress we became aware of complementary results, which had been observed during work carried out independently at Berkeley.[13]

Since the preliminary results were presumptive evidence that **1** was an indole alkaloid biosynthetically, further work was carried out designed to permit a distinction to be made between the corynantheidine–geissoschizine and lactam hypotheses. An insignificant to zero incorporation of radioactivity into **1** was observed on feeding [aryl-^3H]-**2b**. This result, considered in light of the apparently successful incorporation of at least one of the epimers of **4**, seems to rule out the corynantheidine–geissoschizine hypothesis, for **2b** has been established as a precursor of *Corynanthe*-type indole alkaloids of *C. roseus*,[14] although not necessarily by a direct route from **4b**. On the other hand, since **4b**, *but not* **4a**, has been shown to be the penultimate precursor of the entire carbon skeleton of other indole alkaloids,[9] and since the cyclization **4a** to **5a** and **4b** to **5b** does not occur with C-3 epimerization,[9,15] it was surprising that we observed only insignificant incorporation of radioactivity from [5-$^3H_2$]- *or* [14-$^3H_2$]-**5b**[12] into **1**. Our lactam hypothesis was saved from ignominious defeat by the subsequent observation that [5-$^3H_2$]-, [14-$^3H_2$,5-^{14}C]-, and [14-$^3H_2$,5-^{14}C]-18,19-dihydro-**5a**[12] were incorporated quite efficiently into **1** (Table I).

Table I. Relative Incorporation of Radioactivity into **1** from Precursor Feeding Experiments

Precursor	Percentage absolute incorporation[a]	Percentage specific incorporation[b]
[2-^{14}C]Tryptophan	0.004	0.009
3(RS)-[2-^{14}C]Mevalonate	0.005	
[6-$^3H_2$]-**3**	0.004	0.02
3(RS)-[5-$^3H_2$]-**4**	0.04	
3(RS)-[14-$^3H_2$]-**4**	0.04	
[5-$^3H_2$]-**5a**	1.4	1.8
[14-$^3H_2$,5-^{14}C]-**5a**	2.0	1.3
($^3H/^{14}C$ = 11.5)	($^3H/^{14}C$ = 10.9, 95% ^3H retention)	
18,19-Dihydro[14-$^3H_2$,5-^{14}C]-**5a**	4.7	0.8
($^3H/^{14}C$ = 11.4)	($^3H/^{14}C$ = 10.6, 93% ^3H retention)	
[5-$^3H_2$]-**5b**[c]	0.004	
[14-$^3H_2$]-**5b**	0.001	
[aryl-$^3H_4$]-**2b**[d]	0.005	
[3,6-$^3H_3$]Loganin (**11**)[e]	0.01	

[a] Total dpm isolated divided by total dpm fed × 100. [b] Dpm/mmol isolated divided by dpm/mmol fed × 100. [c] Three separate feeding experiments carried out with parallel feedings of **11** or **5a**. [d] Three separate feeding experiments carried out with parallel feedings of tryptophan. **4**, **5a**, and/or **5b**. [e] Prepared according to A. R. Battersby, C. R. Hutchinson, N. D. Westcott, and R. A. Larson, manuscript in preparation. Presented in part at the 163rd National Meeting of the American Chemical Society, Boston, Mass., 1972, ORGN 11. A Kuhn–Roth degradation was used to show that 44% of the radioactivity of **1** was located at C-19, in excellent agreement with the anticipated value of 45% (**11** contained 45% of its tritium label at C-3).

Due to the paucity of suitable degradative chemistry, we turned to ^{13}C nmr spectroscopy to obtain the necessary corroboration of the site-specific incorporation of **5a** into **1**. To our knowledge this represents the first demonstration of the suitability of carbon magnetic resonance (cmr) spectroscopy for biosynthetic studies in higher plants.[16]

(12) Prepared analogously to methods described in (a) ref 9; (b) A. R. Battersby and R. J. Parry, *Chem. Commun.*, 31 (1971).

(13) Professor H. Rapoport, personal communication, for which we are grateful.

(14) A. R. Battersby and E. S. Hall, *Chem. Commun.*, 793 (1969).

(15) G. N. Smith, University of Manchester, personal communication.

(16) Successful application of cmr spectroscopy in a study of the biosynthesis of colchicine has been obtained recently: A. R. Battersby, P. W. Sheldrake, and J. Milner, personal communication.

A proton-decoupled, natural abundance cmr spectrum of **1** in DMSO-d_6 was run at 50°. All but the carbonyl carbon signals were found and the chemical shifts assigned (Table II). Analysis of the quinoline

Table II. Cmr Data for **1**[a]

Carbon	Chemical shift[b]	Relative peak height (Natural abundance[c])/ (^{13}C-enriched[d])
2	156.8	
3	145.4[e]	
5	50.2	97/150
6	129.7	
7	131.4[f]	
8	127.9	
9	128.4	
10	127.5	
11	129.0	
12	130.2[f]	
13	149.9[e]	
14	96.7	113/90
15	147.9[e]	
16	119.0	
17	65.4	100/100
18	7.8	77/63
19	30.6	103/87
20	72.4	52/55

[a] Determined on a JEOL PFT-100 nmr spectrometer at 25.1 MHz; 16K data points (time domain) over 5000 Hz were used with 3.2 and 5.0 sec pulse repetition rates and 90° pulses. [b] Relative to external TMS: $\delta^{DMSO} = \delta^{TMS} + 39.6$ ppm. [c] *Ca.* 0.1 *M*. [d] *Ca.* 0.03 *M*. [e] These three assignments could be interchanged. [f] These two assignments may be interchanged.

and α-pyridone nuclei was based on the shift data of quinoline,[17] α-pyridone,[18] and its *N*-methyl derivative[18] and application of proper substituent effects.[19] The methines of the benzene half of the quinoline moiety were assigned in the order in which they appear in quinoline itself, the methine of the pyridine half being 5 ppm upfield of the corresponding quinoline carbon resonance. Carbons 2 and 6 are displaced downfield by 7 and 10 ppm, respectively, from their normal quinoline resonances in agreement with shift differences evaluated from a methylated quinoline model.[17] The small shift difference (0.8 ppm) between C-16 of **1** and C-3 of α-pyridone and the downfield shift of C-15 fit the known effect of alkylation of the α- and β-carbons of conjugated ketones.[19b] There being only one methyl group and one quaternary carbon and each methylene unit being surrounded by a different environment, all nonaromatic carbons of camptothecin are unique.

[1-^{13}C]Tryptamine was synthesized from [^{13}C]KCN and thereby [5-^{13}C]-**5a** and **5b** were obtained by procedures analogous to those reported by Battersby and coworkers.[10,12b] The resulting labeled **5b** contained ≧84 atom % ^{13}C as shown by mass spectrometric analysis, being labeled solely at C-5 (cmr, δ 40.9). Intact plants (206 g) were wick-fed [5-^{13}C]-**5a** (38 mg) over a 2-day period and then maintained under growth lamps[20] at 27° for an additional 17 days during December, 1973. Standard isolation techniques were used to obtain chromatographically pure **1** (20 mg) from the whole plants. The proton noise-decoupled cmr spectra of natural-abundance and "enriched" **1** were determined under as closely identical conditions as possible to obtain comparable S/N ratios and peak heights for the relevant carbons. The normalized peak heights of six of the carbons of **1** (Table II) were compared, particular significance being given to the C-5 and C-17 methylenes. It could be seen that, as anticipated from consideration of the lactam hypothesis, *only* C-5 of **1** had been labeled by the biosynthetic incorporation of [5-^{13}C]-**5a**. The signal enhancement of C-5, corresponding to *ca.* 55% increase over natural abundance (C-5 compared to C-17), represents a specific incorporation of *ca.* 1.6%, quite in line with the specific incorporation of 1.3–1.8% of radioactively labeled **5a**.

It is noteworthy that from the results of our biosynthetic study of **1**, it appears that there are two closely related pathways for indole alkaloid biosynthesis among higher plants. Both pathways involve the utilization of only one epimer of a common key intermediate, **4**, yet both epimers of **4** are produced by *C. acuminata*[21] and *C. roseus*.[9] An intriguing question arises from these and other observations herein; are some steps of biosynthetic pathways to secondary natural products more the result of chemical reactivity under optimal conditions and less the outcome of stereoselective, enzymatic control? Similar views have been expressed recently by Scott and Wei[22] for the Vinca alkaloids.

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(17) L. F. Johnson and W. C. Jankowski, "Carbon-13 NMR Spectra," Wiley, New York, N. Y., 1972.
(18) U. Vogli and W. von Philipsborn, *Org. Magn. Resonance*, **5**, 551 (1973).
(19) (a) J. B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, N. Y., 1972; (b) G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists," Wiley, New York, N. Y., 1972.
(20) Westinghouse Plant-Gro F40.
(21) Shown by a cold-trap experiment in which the two epimers of **5** were added to the plant material from a feeding experiment with [3,6-^{3}H$_2$]loganin. Under the isolation conditions, **4b** was converted to **5b**. The reisolated **5a** contained 0.01% of the total radioactivity fed and **5b**, 0.03%.
(22) A. I. Scott and C. C. Wei, *J. Amer. Chem. Soc.*, **94**, 8264 (1972).

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Dipolar Relaxation in Shift Reagents as a Solution Structural Probe

Sir:

The basis for effecting meaningful solution structural determinations using proton nmr shifts due to lanthanide shift reagents,[1] SR's, is the assumed magnetic axial symmetry which permits the analysis of the dipolar

(1) R. E. Sievens, Ed., "Nuclear Magnetic Resonance Shift Reagents," Academic Press, New York, N. Y., 1973.