Table I. Purine Synthesis from 6-Amino-5-phenylazopyrimidines and Aryl Aldehydes

Starting material	Aryl aldehyde	Product[a]	Yield (%)
Ic	Benzaldehyde	IIIe	70
Ic	*p*-Chlorobenzaldehyde	IIIf	83
Ic	3,4-Dichlorobenzaldehyde	IIIg	95
Ic	*p*-Anisaldehyde	IIIh	78
Ic	*p*-Dimethylaminobenzaldehyde	IIIi	93
Id	Benzaldehyde	IIIj	70
Id	3,4-Dichlorobenzaldehyde	IIIk	78

[a] None of products melted below 330°.

Table II. Pyrazolo[3,4-*d*]pyrimidine Synthesis from 6-(Benzylidenehydrazino)-1,3-dimethyluracil and Aryl Aldehydes

Starting material	Aryl aldehyde	Product	Mp, °C	Yield (%)
VIIa	Benzaldehyde	VIIIa	193	83
VIIb	*p*-Chlorobenzaldehyde	VIIIb	181	87
VIIc	3,4-Dichlorobenzaldehyde	VIIIc	194	85
VIId	*p*-Anisaldehyde	VIIId	160	84
VIId	*p*-Chlorobenzaldehyde	VIIIe	173	75
VIIe	Benzaldehyde	VIIIf	326	67

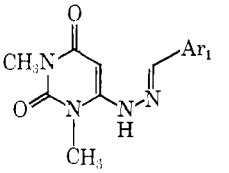
theophylline (IIId) (mp >330°, 60%) and 7-(*p*-chlorophenyl)-1,3-dimethyl-5-phenyl-5,6-dihydro-6-azalumazine (IVd) (mp 250°, 15%).

This new purine synthesis appears to be general and is equally applicable to other 6-amino-5-phenylazopyrimidines. Namely, fusion of 6-amino-1-methyl-5-phenylazouracil (Ic) and 6-amino-4-hydroxy-2-phenyl-5-phenylazopyrimidine (Id) with excess aryl aldehydes under the same conditions gave the corresponding purine derivatives (IIIe–k) in good yields (see Table I). In these cases, the corresponding 6-azalumazine derivatives were not obtained.

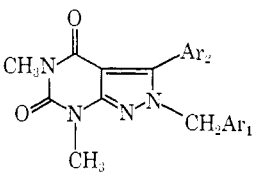
Next, we have used 5-benzylidene derivatives of 6-(benzylidenehydrazino)uracils for this reaction. The refluxing of 6-(benzylidenehydrazino)-1,3-dimethyluracil (VIIa)[6] with a slight excess of benzaldehyde in dimethylformamide for 3 hr gave exclusively 2-benzyl-5,7-dimethyl-3-phenylpyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,7*H*)-dione (VIIIa) in excellent yield. Similarly, the heating of other 6-(benzylidenehydrazino)-1,3-dimethyluracil derivatives (VIIb–e)[6] with several aryl aldehydes in dimethylformamide gave the corresponding 3-aryl-2-benzylpyrazolo[3,4-*d*]pyrimidine derivatives (VIIIb–f) (see Table II). The structures of VIII were established by comparison with authentic samples prepared by the benzylation[7] of 3-aryl-5,7-dimethylpyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,7*H*)-diones[6] with the corresponding benzyl halides in dimethylformamide in the presence of potassium carbonate. Although we did not detect any other compounds in the reactions, the possible intermediates must be 5-benzylidene derivatives (IX)[8] of VII in consideration of the products and of the next reaction described below.

5-Benzylidene-6-(benzylidenehydrazino)-3-methyluracil (Xa) (mp 277–278°) and 5-(*p*-chlorobenzylidene)-6-(*p*-chlorobenzylidenehydrazino)-3-methyluracil (Xb) (mp 298°), which were prepared by the condensation of the corresponding 6-(benzylidenehydrazino)-3-methyluracils and aryl aldehydes in ethanol at 90°, were heated under reflux in dimethylformamide for 8 hr; dilution with ethanol caused separation of 2-benzyl-5-methyl-3-phenyl- (XIa) (mp 229–230°, 68%) and 2-(*p*-chlorobenzyl)-3-(*p*-chlorophenyl)-5-methyl-pyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,7*H*)-diones (XIb) (mp 267°, 72%), respectively. Their structures were confirmed by the transformation of XIa and XIb into VIIIa and VIIIb by the methylation with methyl iodide in dimethylformamide in the presence of potassium carbonate. The refluxing of the 6-(benzylidenehydrazino)-3-methyluracils with aryl aldehydes in dimethylformamide gave directly XI (Scheme II).

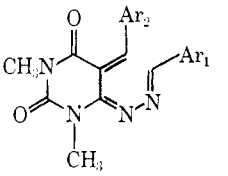
Scheme II



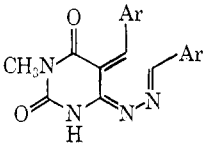
VIIa, $Ar_1 = C_6H_5$
b, $Ar_1 = p\text{-}ClC_6H_4$
c, $Ar_1 = 3,4\text{-}Cl_2C_6H_3$
d, $Ar_1 = p\text{-}CH_3OC_6H_4$
e, $Ar_1 = p\text{-}(CH_3)_2NC_6H_4$



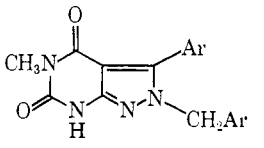
VIIIa, $Ar_1 = C_6H_5$; $Ar_2 = C_6H_5$
b, $Ar_1 = p\text{-}Cl\text{-}C_6H_4$; $Ar_2 = p\text{-}ClC_6H_4$
c, $Ar_1 = 3,4\text{-}Cl_2\text{-}C_6H_3$; $Ar_2 = 3,4\text{-}Cl_2C_6H_3$
d, $Ar_1 = p\text{-}CH_3O\text{-}C_6H_4$; $Ar_2 = p\text{-}CH_3OC_6H_4$
e, $Ar_1 = p\text{-}CH_3OC_6H_4$; $Ar_2 = p\text{-}ClC_6H_4$
f, $Ar_1 = p\text{-}(CH_3)_2N\text{-}C_6H_4$; $Ar_2 = C_6H_5$



IX



Xa, Ar = C_6H_5
b, Ar = $p\text{-}ClC_6H_4$



XIa, Ar = C_6H_5
XIb, Ar = $p\text{-}ClC_6H_4$

These new cyclizations may involve a possible [$\pi 4 + \pi 2$] cycloaddition of azalogs of hexatriene, followed by thermal 1,5 shift of a hydrogen atom to give the respective heterocycles. The syntheses of other heterocycles by this route are currently under investigation.

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(6) F. Yoneda and T. Nagamatsu, *Synthesis*, 300 (1973).
(7) In the benzylation, the isomeric 1-benzylpyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,7*H*)-dione derivatives were obtained as the minor products
(8) Attempts to obtain IX by the condensation of VII with aryl aldehydes at lower temperatures were unsuccessful, with the starting materials being recovered.

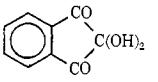
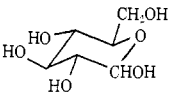
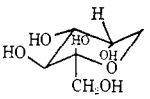
Tight Binding of Hydroxyl Protons in *gem*-Diols and Hemiacetals

Sir:

The hydroxyl protons of *gem*-diols and hemiacetals are located in a tighter binding potential than are

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

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).

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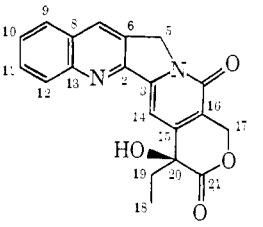
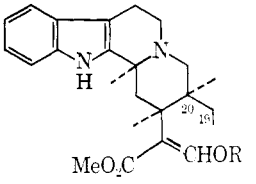
Biosynthesis of Camptothecin. I. Definition of the Overall Pathway Assisted by Carbon-13 Nuclear Magnetic Resonance Analysis[1]

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).