Å, respectively, whereas the bridging carbonyl Co-C and C-O bonds vary from 1.928 (8) to 1.959 (9) Å and from 1.157 (9) to 1.164 (9) Å, respectively. These trends are in accord with those found in other metal carbonyl clusters. (4) The P-C(phenyl) bond length of 1.81 Å is that expected for a single-bond distance. No assessment from bond-length correlations of the differences in Co-P bonding between the five-coordinate phosphorus atoms in $Co_4(CO)_8(\mu_2\text{-}CO)_2(\mu_4\text{-}PC_6H_5)_2$ and four-coordinate phosphorus atoms in other cobalt clusters is made at this time, partly due to a present lack of structural data for RP-bridged cobalt trimers.

An examination of Table I shows that the major alteration in the Co_4E_2 core upon a replacement of the two sulfur atoms by PC_6H_5 ligands is the large decrease in the E···E distance from 2.74 (2) Å[12,13] for E = S to 2.544 (3) Å for E = P. Because of the close agreement between the covalent radii of S and P (and likewise between their van der Waal radii)[14] and because these structurally analogous molecules do not appear to exhibit unusual steric effects due to interligand overcrowding, a plausible explanation in our opinion for the 0.20 Å shortening of the E···E distance (to a value only 0.3 Å greater than an accepted single-bond P-P distance)[15] lies in the two P atoms being pulled toward each other due to attractive bonding interactions which may involve contributions from the phosphorus 3d orbitals. Since the Co-E bond lengths are essentially identical in these Co_4P_2 and Co_4S_2 cores, the observed deformation in the Co_4P_2 core to give the smaller E···E distance necessitates the concomitant increases in the corresponding Co-Co bond distances and corresponding Co-E-Co bond angles. Similar short P···P separations, which are 0.3 Å shorter than the corresponding S···S separations, exist in other types of organometallic clusters—viz., $Fe_3(CO)_9(\mu_3\text{-}PC_6H_5)_2$ (P···P, 2.592 (3) Å)[3] vs. $Fe_3(CO)_9(\mu_3\text{-}S)_2$ (S···S, 2.885 (2) Å)[4b] and $Co_4(\eta^5\text{-}C_5H_5)_4(\mu_3\text{-}P)_4$ (P···P, 2.57 Å)[16] vs. $Fe_4(\eta^5\text{-}C_5H_5)_4(\mu_3\text{-}S)_4$ (S···S, 2.88 Å).[17] Although the possibility of residual P···P bonding was considered in the latter cubane-like molecule, it was concluded that the "extremely short P···P contacts are primarily nonbonding" due to the overall geometrical constraint imposed on the phosphorus atoms by the other atoms.[16] On the basis of the results presented here, we now propose that the short P···P distances in these organometallic clusters instead arise from distinct bonding forces which (despite being small relative to metal-ligand interactions) do nevertheless cause a considerable perturbation on the geometry; the much smaller P···P distances also imply considerably stronger P···P than S···S interactions for each of the above types of organometallic clusters.

The work presented here will be reported in full upon completion of complementary research which will include physical-chemical studies of related species as well as the application of the nonparameterized Fenske-Hall MO method[18] in order to provide an assessment of our bonding conclusions.

Acknowledgments. We are very grateful to the National Science Foundation (No. GP-19175X) for their financial support. The use of the UNIVAC 1110 computer at the Academic Computing Center, University of Wisconsin (Madison), was made available through partial support of the National Science Foundation and the Wisconsin Alumni Research Foundation administered through the University Research Committee.

Supplementary Material Available. A listing of atomic coordinates will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Business Office, Books and Journals Division, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.00 for photocopy or $2.50 for microfiche, referring to code number JACS-75-6904.

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(9) $Co_4(CO)_8(\mu_2\text{-}CO)_2(\mu_4\text{-}PC_6H_5)_2$: triclinic; $P\bar{1}$; a = 11.390 (6), b = 14.257 (10), c = 9.124 (6) Å; α = 97.99 (6), β = 113.27 (4), γ = 96.13 (5)°; V = 1326.4 Å^3; d_{calcd} = 1.83 g cm^{-3} for Z = 2. Least-squares refinement of the coordinates of all atoms with variable anisotropic thermal parameters for the nonhydrogen atoms and fixed isotropic ones for the hydrogen atoms gave $R_1(F)$ = 4.3% and $R_2(F)$ = 6.5% for 2661 independent diffractometry data ($I \geq 2\sigma(I)$).
(10) For computation of distances and bond angles, atomic coordinates will appear following these pages in the microfilm edition of this volume of the journal.
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Biosynthesis of the Indole Alkaloids. A Cell-Free System from *Catharanthus roseus*

Sir:

From the inception of experimental analysis of hypotheses for the biogenesis of the plant alkaloids it has been recognized[1] that problems of permeability,[2] compartmentation of metabolic pools,[3] and translocation phenomena[4] have imposed severe limitations on incorporation levels of labeled substrates in feeding studies with the intact higher plant. These factors seem to be particularly dominant in those species which produce complex indole alkaloids, where the reported specific incorporations of labeled "intermediates" are mainly in the range 10^{-1}–10^{-3}%.[5,6] Notwithstanding considerable progress in the delineation of the broad outlines[5,7] of alkaloid biosynthesis in *Catharanthus*, *Vinca*, and *Aspidosperma* spp., there would appear to be general agreement[1,4,5] that only with the advent of enzymological techniques for alkaloid synthesis could those criteria recently summarized by Cornforth[8] be met for the many postulated biointermediates on the tryptophan-secoiridoid pathway leading to the major alkaloid families.

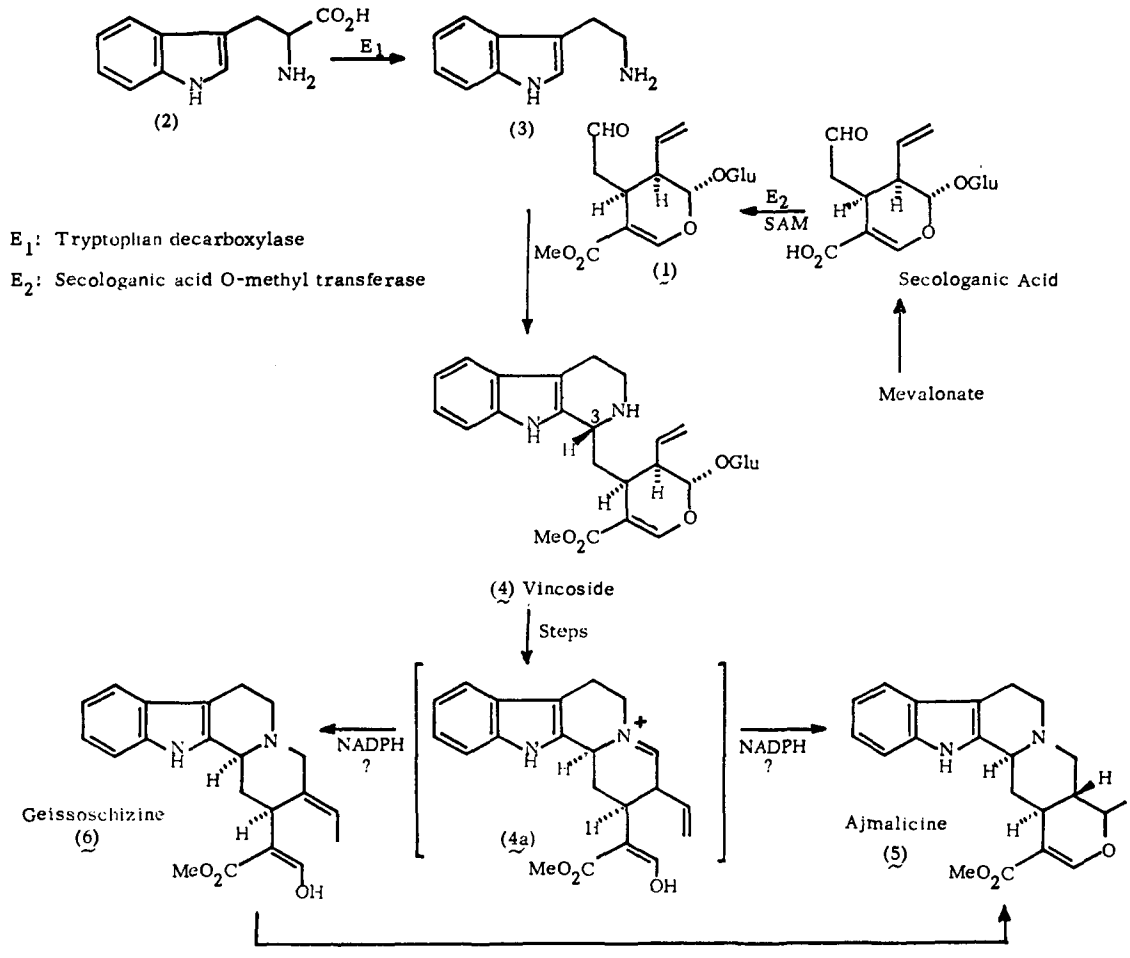

Table I[e]

Expt	Preparation	Substrate	Alkaloids synthesized (% incorp)
1	A[a]	[2-^{14}C]Tryptamine Secologanin (1)	Ajmalicine (5) (0.5%) Geissoschizine (6) (<0.1%)
2	B[b]	[2-^{14}C]Tryptamine Secologanin (1)	Ajmalicine (18%) Geissoschizine (1%)
3	B[c]	Tryptamine [OC-$^{3}H_3$]Secologanin (1)	Ajmalicine (2.8%) Geissoschizine (0.21%)
4	B[d]	[Ar-^{3}H]Geissoschizine (6)	Ajmalicine (7.7%) + several *unknown* metabolites

[a] Incubation contained 250 nmol of FAD, 250 nmol of NADPH[f], 2.5 μmol of secologanin, 2.5 μCi/0.53 μmol of [^{14}C]tryptamine–HCl and 24 mg of protein in a total volume of 12 ml. [b] Incubation contained 250 nmol of FAD, 250 nmol of NADPH, 2.5 μmol secologanin, 2.5 μCi/0.53 μmol of [^{14}C]tryptamine–HCl, and 19.2 mg of protein in a total volume of 12 ml. [c] Incubation contained 2.5 μmol of tryptamine–HCl, 11.1 μCi/4.27 μmol of [^{3}H]secologanin, 0.5 μmol of NADPH, and 6.4 mg of protein in a total volume of 4 ml. [d] Incubation contained 0.5 μmol of NADPH, 14 μCi/0.2 μmol of [^{3}H]geissoschizine (in acetate form), and 6.4 mg of protein in a total volume of 4 ml. [e] All incubations were carried out in 0.05*M* Tris–maleate buffer at pH 7.0, containing 10 m*M* β-mercaptoethanol. Boiled enzyme solutions did not show any natural alkaloids other than the in vitro coupling of tryptamine and secologanin, forming vincoside and isovincoside. This coupling was not appreciably affected by β-mercaptoethanol. [f] The enzyme solutions were not dialyzed to eliminate endogenous NADPH. With addition of NADPH, there seemed to be a slight increase in the incorporation. The essentiality of NADPH for the enzyme reaction is now under investigation.

Some initial progress towards this goal has been made by the isolation from *C. roseus* of an *O*-methyl transferase which catalyzes the conversion of loganic and secologanic acids to their methyl esters, loganin and secologanin (**1**), respectively.[9] Previous attempts[10] to detect complete alkaloid synthesis with acetone powders from *C. roseus* homogenates utilizing secologanin (**1**) and tryptophan (**2**) were unsuccessful, although the formation of tryptamine (**3**) from **2** mediated by tryptophan decarboxylase[11] (E 4:1:1) could be reproducibly demonstrated. In this communication we wish to report the cell-free biosynthesis of the first major class of indole alkaloid[12] (*Corynanthé*) from secologanin (**1**) and tryptamine (**3**) using a mixture of soluble enzymes obtained from seedlings (preparation A) and tissue cultures (preparation B) of *C. roseus*.

For the preparation of crude enzyme solution (A), a suspension of 5–7 day old seedlings[10] (4.7 g) in Tris–maleate buffer (0.05 *M*; pH 7.0) containing Polyclar AT (4.7 g) was homogenized (Sorvall omnimixer at maximum speed for 2 min). The homogenate was centrifuged at 37000*g* for 20 min, and the supernatant was used for incubations, which were carried out for 2 hr at 34°C (see Table I).

Calluses[13,14] from *Catharanthus roseus* (*Vinca rosea*) stems and leaves were homogenized in 0.05 *M* Tris–maleate buffer at pH 7.0, containing 10 m*M* β-mercaptoethanol (callus:buffer = 1:4 w/v). Acid-washed Polyclar AT was added to the homogenate in the proportion of 0.5 g/g of callus. After centrifugation at 37000*g* for 20 min, the supernatant preparation (B) obtained was incubated with substrates and cofactors (see Table I) for 2 hr at 34°C. The products in each case were extracted with $CHCl_3$, first at pH 7.0, then at pH 9.0 (adjusted with NH_4OH). The combined $CHCl_3$ extract was cochromatographed two-dimensionally with reference alkaloids (first in $CHCl_3$–MeOH, 9:1, then ether) on Silplate F22 (Brinkman). Autoradiography and recrystallization with authentic alkaloids showed that ajmalicine (**5**) (18% incorporation) and geissoschizine

(6) (1% incorporation) were synthesized from [^{14}C]tryptamine and secologanin.[15] [Ar-^{3}H]Geissoschizine was metabolized into ajmalicine (7.7%) and several other alkaloids, the identities of which are under investigation. The formation of akuammicine and stemmadenine was not detected under these conditions.

The following conclusions can be drawn from the data of Table I. (a) A complete system of soluble enzymes is present in the 37000*g* supernatant fraction which catalyzes the formation of the *Corynanthé* alkaloids from tryptamine and secologanin in presence of NADPH, thiols, and Tris buffer. (b) The necessary reductive step between the postulated intermediate (**4a**) (derived in turn from vincoside (**4**)) and ajmalicine (**5**) or geissoschizine (**6**) and the later members appears to depend on NADPH. (c) The role of tryptamine and secologanin as true precursors has been established at the cell-free level. (d) The use of callus tissue allows the isolation of a particularly active synthetase mixture and offers considerable advantages over young seedlings as the source of biological material; cf. the relative incorporations of tryptamine into ajmalicine (preparation A, 1%; preparation B, 18%) and of geissoschizine (**6**) into ajmalicine (whole plants, 0.12%,[16] 0.22%;[5c] preparation B, 7.7%). (e) Large scale, high yielding incubations can now be used to define (by isolation) the chemical structure of the various intermediates and metabolites already detected by autoradiography. (f) Purification and immobilization of selected enzymes of the pathway are now feasible.

Acknowledgment. We wish to thank the National Institues of Health (Grant CA 11095) for generous support of this work and Dr. W. Fitch and Mr. A. Brown for help with the growing of callus tissue.

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Preparation of an Optically Active Prostaglandin Intermediate via Asymmetric Induction

Sir:

We describe herein an improved method for the preparation of the key prostaglandin intermediate **1**[1] in optically pure form *without resolution* by a process which utilizes a new, readily accessible, recyclable, and efficient chiral controlling group (Scheme I).

Treatment of the optically pure acrylate **2**, $[\alpha]^{23}D$ +16.21° (*c* 1.68, CH_2Cl_2), with 0.7 equiv of aluminum chloride[2] in methylene chloride at −55° for 1 hr followed by the addition of 2.5 equiv of 5-benzyloxymethylcyclopentadiene[1a] at −55°[3] affords an 89% yield of the endo adduct **3**[4,5] as an oil, $[\alpha]^{23}D$ −21.3° (*c* 2.2, $CHCl_3$).

That the absolute configuration of **3** is as shown has been proven by conversion to the known, optically active iodolactone (**1**) and agrees with the stereochemical prediction made on the basis of Walborsky's work with *R*-(−)-men-

Scheme I