One explanation for this increased stability could be due to hydrogen bonding between the H (D) and an oxygen in the metal chelate. Examination of molecular models of the verbenols and verbanols suggests that the metal coordinates to the hydroxyl oxygen in a nearly eclipsed conformation relative to the geminal hydrogen. The H–C–O–Eu torsional angle that results from a treatment similar to that of Briggs and coworkers[6] is approximately 30°. This conformation, sterically required in the verbenols and verbanols, places the geminal hydrogen above one of the dipivalomethanato chelate rings of the metal complex, in a position which may facilitate hydrogen-bond formation to one of the chelate oxygen atoms. If such hydrogen bonding is stronger for deuterium than for hydrogen, then increased stability would result. Another possible explanation is an increase in base strength of the alcohol oxygen due to the deuterium substitution.[7]

Shifts induced by tris(dibenzoylmethanato)europium(III), $Eu(DBM)_3$, and tris(1-benzoylacetonato)europium(II), $Eu(BAT)_3$,[8] also indicate preferential association by the labeled compounds. The per cent difference of shift between light and heavy molecules was slightly greater than those obtained from $Eu(DPM)_3$-shifted spectra in the case of $Eu(DBM)_3$ and virtually identical in the case of $Eu(BAT)_3$-shifted resonances. These results suggest a chelate ring substituent effect on hydrogen-bond formation and show that the nature of the ligands influences the relative strengths of the hydrogen bond.

Shifts induced in the nmr spectra of *cis*-verbenol by $Eu(DPM)_3 \cdot 2py$, $Eu(DPM)_3$, and $Pr(DPM)_3$ were compared. After normalization to equivalent total shift magnitudes, the pattern of shifts induced by each of the metal complexes was similar. Consequently, the effective magnetic symmetries[6] (*i.e.*, the principle axis and associated angle dependences) of the different metal chelate–substrate complexes are similar.

(6) J. Briggs, F. A. Hart, and G. P. Moss, *Chem. Commun.*, 1506 (1970).
(7) Suggested by referees.
(8) Although relatively insoluble,[2c] these chelates may be dissolved in carbon tetrachloride by the addition of a few drops of pyridine, and usable shifts may be obtained.

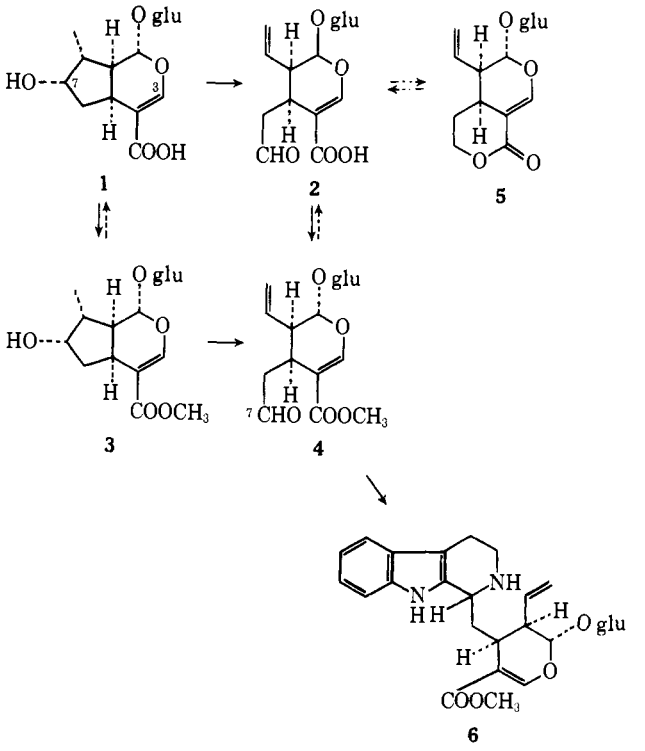
Gerard V. Smith,* Walter A. Boyd, C. C. Hinckley*
Department of Chemistry and Biochemistry
Southern Illinois University Carbondale, Illinois 62901
Received June 30, 1971

Occurrence and Biosynthesis of Secologanic Acid in *Vinca rosea*[1]

Sir:

In the biosynthesis of indole alkaloids of *Vinca rosea*, the cyclopentanoid ring of loganin (**3**) is cleaved to afford secologanin (**4**) with the generation of an aldehyde at C-7 (Scheme I).[2,3] This aldehyde undergoes Mannich condensation at the α position of tryptamine to yield the β-carboline, vincoside[4] (**6**). Sweroside (**5**), another constituent of *V. rosea*, has been shown to be a precursor of the indole alkaloid, vindoline, although its C-7 is at the alcohol oxidation level.[5] We have postulated that metabolism of sweroside (**5**) proceeds *via* secologanic acid[6] (**2**) and we wish to report evidence to support this hypothesis.

Scheme I



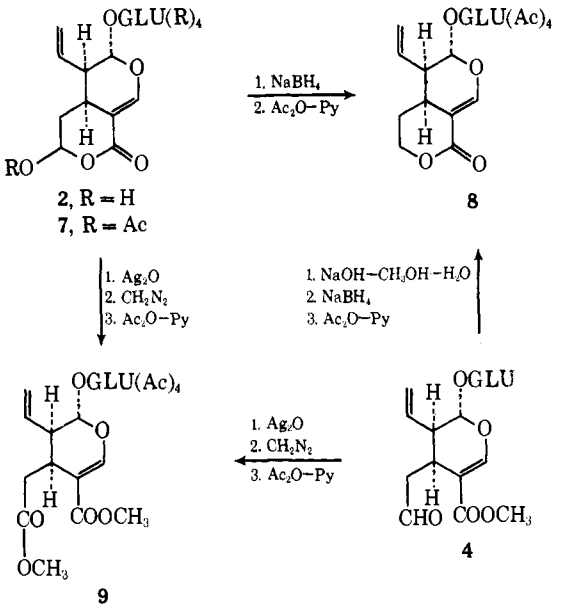
1 2 5 3 4 6

In examining the acidic constituents of *V. rosea*, two new secoiridoid glucosides have been isolated. Gradient elution ion exchange chromatography of the methanolic extract of *V. rosea* afforded an amorphous compound identified as secologanic acid (**2**): optical rotation $[\alpha]D$ −115° (*c* 1, CH_3OH); uv λ_{max}^{EtOH} 239 nm (log ϵ 3.97); ir λ_{max}^{Nujol} 1622 cm^{-1}; nmr[7] (D_2O) δ 9.9 (t, CHO, H-7), 7.17 (d, H-3). Upon acetylation, an epimeric mixture of the pentaacetyl lactol (**7**) was obtained as a colorless oil: optical rotation $[\alpha]D$ −146° (*c* 1, $CHCl_3$); uv λ_{max}^{EtOH} 243 nm (log ϵ 3.97); nmr ($CDCl_3$) δ 7.55 (d, J = 2.0 Hz, H-3), 6.4 and 6.5 (m, H-7), 5.0–5.5 (m, 8 H's), 4.3 (dd, CH_2 of glucose), 3.7 (m, 1 H), 2.13, 2.10, 2.03, 2.00, 1.98 (each s, $5CH_3CO$).[8–10]

Chemical evidence to complement spectral data is outlined in Scheme II. Secologanic acid afforded sweroside tetraacetate (**8**) having properties identical with reported values.[11,12] Mixture melting points with an authentic sample of **8** (provided by Dr. Linde)

(1) Monoterpene Biosynthesis. V. For part IV, see K. M. Madyastha, R. Guranaccia, and C. J. Coscia, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, **14**, 175 (1971).
(2) A. R. Battersby, R. S. Kapil, J. A. Martin, and L. Mo, *Chem. Commun.*, 133 (1968).
(3) P. Loew and D. Arigoni, *ibid.*, 137 (1968).
(4) A. R. Battersby, A. R. Burnett, and P. G. Parsons, *J. Chem. Soc. C*, 1187, 1193 (1969).
(5) H. Inouye, S. Ueda, and Y. Takeda, *Chem. Pharm. Bull.*, **19**, 587 (1971).
(6) R. Guarnaccia, L. Botta, and C. J. Coscia, *J. Amer. Chem. Soc.*, **92**, 6098 (1970).
(7) Nmr spectra were determined with a Varian A-60 spectrometer.
(8) Secologanic acid pentaacetate obtained by chemical degradation of foliamenthin was characterized by the Arigoni and Battersby groups.[9]
(9) P. Loew, Ch. V. Szczepanski, C. J. Coscia, and D. Arigoni, *Chem. Commun.*, 1276 (1968); A. R. Battersby, A. R. Burnett, G. D. Knowles, and P. G. Parsons, *ibid.*, 1277 (1968).
(10) Satisfactory elemental analyses were obtained for **7** and **9**.
(11) H. Inouye, S. Ueda, and Y. Nakamura, *Tetrahedron Lett.*, 5229 (1966); 4429 (1968).
(12) H. H. A. Linde and M. S. Ragab, *Helv. Chim. Acta*, **50**, 991 (1967).

Scheme II



showed no depression and identical chromatographic mobilities were observed. Sweroside tetraacetate (**8**) was also prepared from **4** isolated from this plant.

Upon methylation and acetylation of the second new acidic constituent of *V. rosea*, secologanoside, a methyl ester tetraacetate **9** was obtained: mp 140.5°; optical rotation $[\alpha]D$ −99° (*c* 1, $CHCl_3$); uv λ_{max}^{EtOH} 230 nm (log ε 4.08); nmr δ 7.4 (d, *J* = 2 Hz, H-3), 5.0–5.5 (m, 8 H's), 4.2 (dd, $-CH_2$ of glucose), 3.73 (s, OCH_3), 3.77 (s, OCH_3), 2.13, 2.06, 2.03, and 1.95 (each s, $4CH_3CO$).[10] Compound **9** was also synthesized from **4** and **2** (Scheme II).

V. rosea plants were fed [2-^{14}C-2-^{3}H]mevalonate and labeled loganic acid (**1**), loganin (**3**), secologanic acid (**2**), and secologanin (**4**) were isolated by adding carrier glucosides (Table I). After purification, derivatives were prepared and recrystallized to constant specific activity. Selective oxidation of the cyclopentano glucosides and the secoiridoids indicated 0.8–1 atom of tritium at C-7 in agreement with previous experiments[13,14] and consistent with incorporation of mevalonate *via* the isoprenoid pathway. The remaining tritium would be expected to be at C-3[13,14] and higher ratios may be attributed to an isotope effect in the earlier occurring hydroxylation of a tritiated C-3 methyl group.[15,16]

Table I. *In Vivo* Tracer Experiments in *V. rosea*

Precursor	Labeled monoterpene	% incorp of ^{14}C	^{3}H/^{14}C atomic ratio Obsd	Calcd
[2-^{3}H-2-^{14}C]MVA				2:1
	Loganic acid	0.25	1.3:2	1.33:2[a]
	7-Oxologanin from loganic acid		0.48:2	0.33:2[a]
	Loganin	0.02	1.3:2	1.33:2
	7-Oxologanin from loganin		0.54:2	0.33:2
	Secologanic acid	0.17	1.6:2[b]	1.33:2
	Secologanoside from secologanic acid		0.68:2[b]	0.33:2
	Secologanin	0.2	1.2:2	1.33:2
	Secologanoside from secologanin		0.49:2	0.33:2

[a] Calculated on the basis of equilibration of the terminal dimethyl groups.[6] [b] The difference in ^{3}H/^{14}C ratios between secologanic acid and other isolated monoterpenes may not be significant. The dilution of secologanic acid led to lower specific activities and less accurate ratios.

Having established the existence of a methyl transferase which converts loganic acid (**1**) to loganin (**3**) *in vitro*[1] we wished to demonstrate this methylation *in vivo*. From experiments with [2-^{14}C]mevalonate in *V. rosea*, labeled loganic acid was obtained and its specific activity (6.75×10^5 dpm/mmol) determined by methylation to **3** and recrystallization to constant activity as the free glucoside and its pentaacetate.[6,14] Pure loganic acid-^{14}*C* (**1**) (20 mg), regenerated by saponification followed by ion exchange chromatography,[13] was fed to *V. rosea* plants which afforded loganin (**3**) (1.1% incorporation), secologanic acid (**2**) (6.7% incorporation), and secologanin (**4**) (8.8% incorporation). A sample of labeled loganin (**3**) (31 mg, 8.05×10^5 dpm/mmol), similarly obtained and administered to *V. rosea*, was converted to secologanic acid (**2**) (2.0% incorporation).

Purification of a methyl transferase from *V. rosea*, capable of methylating loganic acid (**1**) and secologanic acid (**2**) at comparable rates,[17] coupled with the above results implicates both acids in indole alkaloid biosynthesis. A dual pathway (Scheme I) is thus envisaged in which the acids are either converted to sweroside (**5**) or methylated and utilized in indole alkaloid biosynthesis. *In vivo* conversion of loganin (**3**) to secologanic acid (**2**) indicates the existence of esterase activity in the plant. Previous double labeling studies with loganin (**3**) in *Cephaelis ipecacuanha* also suggest this.[18]

Acknowledgment. Financial support from the NSF and NIH is appreciated.

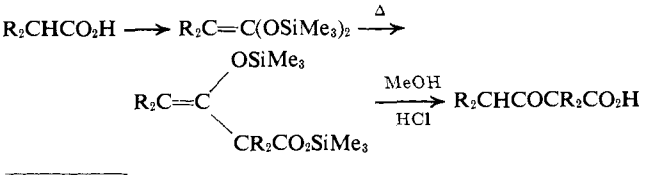
(13) C. J. Coscia, R. Guarnaccia, and L. Botta, *Biochemistry*, 8' 5036 (1969).
(14) C. J. Coscia, L. Botta, and R. Guarnaccia, *Arch. Biochem. Biophys.*, 136, 498 (1970).
(15) J. W. Cornforth, J. W. Redmond, H. Eggerer, W. Buckel, and C. Gutschow, *Nature (London)*, 221, 1212 (1969).
(16) J. Luthy, J. Retéy, and D. Arigoni, *ibid.*, 221, 1213 (1969).
(17) C. J. Coscia, K. M. Madyastha, and R. Guarnaccia, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 30, 1472 (1971).
(18) A. R. Battersby and B. Gregory, *Chem. Commun.*, 134 (1968).

R. Guarnaccia, C. J. Coscia*
Department of Biochemistry
St. Louis University School of Medicine
St. Louis, Missouri 63104
Received August 2, 1971

Reactions of Dianions of Carboxylic Acids with Esters and α,β-Unsaturated Esters, Nitriles, and Aldehydes

Sir:

We have recently discovered a new synthesis of β-keto acids *via* the following reaction scheme.[1] We



(1) Y. N. Kuo and C. Ainsworth, to be published elsewhere.