pling yields. When a portion of the unhydrolyzed peptide solution was chromatographed on the 0.9 × 18 cm PA-35 column (eluted with 0.38 *N* sodium citrate, pH 7.00, at 70 ml/hr), less than 5% free His(Bzl) was present, indicating that removal of noncovalently bound His(Bzl) prior to cleavage was almost complete; thus the His(Bzl) in the hydrolysates came primarily from the peptide. High-voltage paper electrophoresis at pH 1.85 showed a single ninhydrin and chlorine + spot.[12]

These results indicate essentially quantitative reaction between Boc-His(Bzl) and Pro-Phe-O-polymer under the conditions used. No attempts have yet been made to refine the reaction conditions to determine if reaction time or molar excesses of reagents could be reduced without sacrificing yields or optical purity. The tripeptide has been synthesized on a larger (0.5 mmol) scale as a chromatographically homogeneous product in a quantitative yield. As with the analytical scale experiment, the crude product had His (Bzl), Pro, and Phe in equimolar amounts; however, after hydrogenation (48 hr at 30 psi in 10% AcOH in the presence of an equal weight of 10% Pd/C) the Phe content had dropped to 92% and ~8% hexahydrophenylalanine was present, despite the fact that 1–2% His(Bzl) remained. This illustrates an undesirable feature in the use of His(Bzl) in peptide synthesis, the occasional difficulty in selective removal of the benzyl group.

The results described show that 1-hydroxybenzotriazole is effective in reducing racemization during solid-phase peptide synthesis with Boc-His(Bzl) to acceptably low levels without impairing coupling efficiency. It is hoped that this technique will also be useful with histidine analogs, where the use of basicity-suppressing protecting groups for racemization control[4] is not possible.

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(13) This work was supported by Public Health Service Research Grant No. AM 08066.

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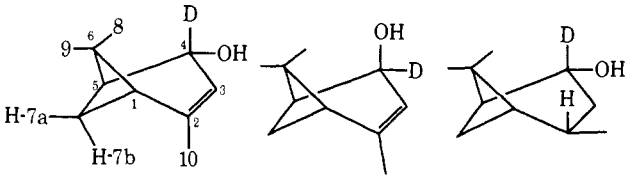
Isotope Effects in Nuclear Magnetic Resonance Spectra Modified by Rare-Earth Shift Reagents

Sir:

Since the original discovery of a rare-earth nuclear magnetic resonance shift reagent,[1] considerable exploration of the field has occurred[2] and new reagents have been found.[3] Although the general theory and utility of the method are well into their formative stages, we wish to report an unusual and novel isotope effect which is not only unexpected, but also discloses new aspects of the nature of the shift reagent-substrate interaction.

In a series of experiments designed to test for the occurrence of [1,3] sigmatropic shifts during catalytic hydrogenation,[4,5] we had occasion to label the verbenols at the alcohol carbon. After a preliminary hydrogenation of a mixture of light and heavy *trans*-verbenol,



1 2 3

4-deuterio-*trans*-verbenol | 4-deuterio-*cis*-verbenol | 4-deuterioverbanol

the verbanol was purified by gas–liquid chromatography and examined by nmr using Hinckley's reagent (the dipyridine adduct of trisdipivalomethanatoeuropium(III), Eu(DPM)$_3$·2py).[1] Concomitant with the expected shifts, a doubling of all hydrogen peaks occurred. The origin of the extra set of peaks was readily apparent when the downfield set increased upon addition of a pure sample of 4-deuterioverbanol to the mixture. Examination of a variety of alcohols and one aldehyde (2-methylbenzaldehyde-d_1, **4**) shows that the effect is quite general (Table I).

Table I. Isotope Effects on Nmr Shifts Induced by Eu(DPM)$_3$·2py

Labeled compd	Resonance obsd	Chemical shift,[a] Hz	Shift changes,[b] Hz	% difference[c]
1	Methyl-8	105	−170	3.1
	Methyl-9	132	−67	3.3
	Olefinic	530	−206	2.9
	H-7a	239	−69	2.7
	H-7b	126	−86	2.6
2	Methyl-8	86	−96	1.6
	Olefinic	530	−361	1.4
	H-7b	130	−408	1.7
4	Methyl	265	−38	4.3

[a] At 100 MHz. Initial chemical shift is same for both labeled and unlabeled compounds. [b] At 100 MHz. Induced changes in shifts are reported for unlabeled material; only enough shift reagent was added in each case to a 50/50 mixture to allow accurate measurement of shift differences between labeled and unlabeled molecules. Per cent difference appears to be independent of shift reagent concentration. [c] (Induced shift of labeled compound − induced shift of unlabeled compound)100/induced shift of unlabeled compound.

This finding indicates a greater association constant between the deuterium-substituted compound and the metal complex than between the light compound and metal complex. Clearly, in addition to simple metal–oxygen association, there exists a contribution to bonding which is enhanced by the presence of a deuterium atom in place of the hydrogen atom geminal to the hydroxyl group.

Variations in the concentration of alcohol, metal complex, and pyridine produced no change in the observed per cent difference for 2-butanol and a number of other alcohols studied. These results suggest that the differences in effective metal chelate–substrate association constants for the light and heavy molecules studied are due principally to differences in metal chelate–substrate complex stability.

(1) C. C. Hinckley, *J. Amer. Chem. Soc.*, 91, 5160 (1969).
(2) (a) C. C. Hinckley, M. R. Klotz, and F. Patil, *ibid.*, 93, 2417 (1971); (b) P. V. Demarco, T. K. Elzey, R. B. Lewis, and E. Wenkert, *ibid.*, 92, 5734 (1970); (c) J. K. M. Sanders and D. H. Williams, *ibid.*, 93, 641 (1971).
(3) (a) G. M. Whitesides and D. W. Lewis, *ibid.*, 92, 6979 (1970); (b) R. E. Rondeau and R. E. Sievers, *ibid.*, 93, 1522 (1971).
(4) G. V. Smith and J. R. Swoap, *J. Org. Chem.*, 31, 3904 (1966).
(5) F. D. Mango, *Advan. Catal.*, 20, 291 (1969).

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.

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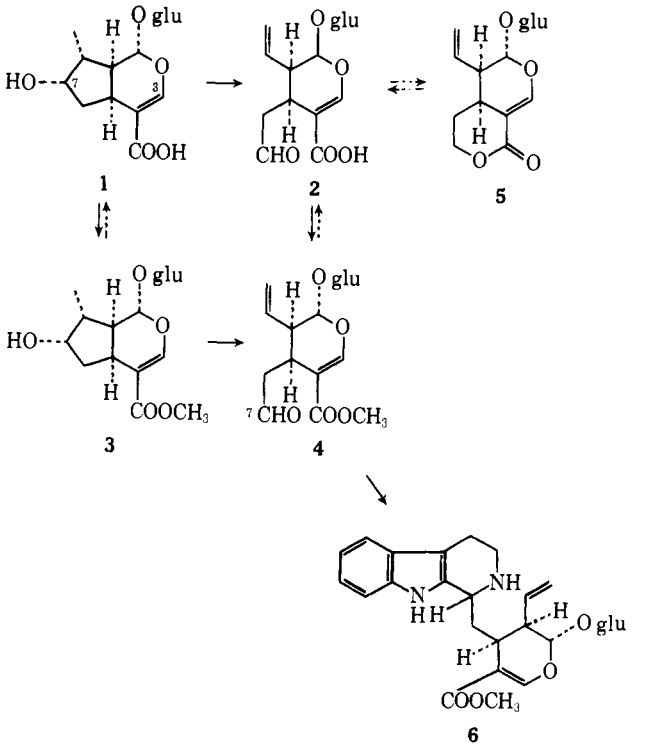
Occurrence and Biosynthesis of Secologanic Acid in *Vinca rosea*[1]

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



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).