cystamine,[12] cysteine–cystine,[13] and lysozyme.[14] These radical anions exhibit λ_{max} ~410 nm with $\epsilon \sim 10^4$ M^{-1} cm^{-1} and are eliminated below pH 4 presumably due to protonation of the anionic form of the substrate or of the radical anion itself. Indeed, we found that the electron adduct to glutathione disulfide at pH 9 showed λ_{max} 420 nm and $\epsilon_{420} > 5 \times 10^3$ M^{-1} cm^{-1}.

The following scheme summarizes our results for glutathione disulfide.

$$\begin{array}{ccc} e_{aq}^- + RSSR & \longrightarrow & (RSSR)^- \\ \downarrow H^+ & & -H^+ \uparrow\downarrow H^+ \\ H + RSSR & \longrightarrow & R\dot{S}SHR \end{array} \quad (5)$$

The H-atom adduct and the protonated form of the electron adduct are apparently identical, with λ_{max} 330 nm and ϵ_{330} 600 M^{-1} cm^{-1} for these nonresonance structures, as compared with the longer wavelength and higher ϵ values for the $(RSSR)^-$ form. We find that this latter species undergoes first-order decay at pH 9–12

$$(RSSR)^- \longrightarrow RS\cdot + RS^- \quad (6)$$

with $k_6 = 2.5 \times 10^5$ sec^{-1} (pH independent). The protonated form exhibits a second-order decay in the presence of *tert*-butyl alcohol with $2k = 2.4 \times 10^9$ M^{-1} sec^{-1}. Unfortunately, any contribution to the decay rate due to the reaction of *tert*-butyl alcohol radicals with RSSHR cannot be excluded at the present time. Note that when both OH radicals and H atoms react with $R\dot{S}SR$, the resulting spectrum (Figure 1b, squares) exhibits the H-atom transient absorption at 330 nm as well as an increased absorption below 290 nm which we attribute to the OH attack.

Observations similar to those reported here have been made for the reaction of H atoms with cysteine–cystine, and the detailed results will be published in the near future.[15]

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Monoterpene Biosynthesis. III. Occurrence and Biosynthesis of Loganic Acid in Indole Alkaloid Synthesizing Plants

Sir:

Within the dicotyledonous angiosperms, the iridoid glucosides have been found in a broad diversity of structural types from which a general biogenetic pathway has not yet emerged.[1] It has been recognized, however, that methyl cyclopentano monoterpene glucosides which are hydroxylated at C-7 serve as intermediates in the biosynthesis of both secoiridoids[2] and indole alkaloids[3] of higher plants of the *Gentianaceae*, *Loganiaceae*, and *Apocynaceae* families. Hence Battersby observed that of three iridoids tested, monotropeine, genipin, and loganin, the latter alone acted as a precursor of the nontryptamine moiety of the indole alkaloids.[3] In retrospect this is also consistent with the congener relationship of loganin and strychnine in *Strychnos nux vomica* fruit.[4] We now have evidence for the occurrence and biosynthesis of the related iridoid, loganic acid, in the indole alkaloid synthesizing plants *Vinca rosea* and *Strychnos nux vomica*.

Seeds of both *Vinca rosea* and *Strychnos nux vomica* were found to contain relatively large quantities of loganic acid, accounting for 1% of their total weight. This concentration is considerably higher than the 0.05% distribution on a fresh weight basis observed for loganic acid in mature *Vinca* plants. Derivatives of the loganic acid from both plants possessed superimposable spectra with those of loganic acid isolated from *Swertia caroliniensis*.[6] This included optical rotation, uv, ir, nmr, and mass spectrometry. Melting points of admixtures of methylated loganic acid or loganin pentaacetate with authentic samples (provided by J. Wolinsky of Purdue University, Lafayette, Ind.) showed no depression.

Small amounts of loganin could also be isolated from *Strychnos* seeds whereas its presence in *Vinca* was ascertained[3] by isotope dilution studies (0.01% incorporation of mevalonate-2-^{14}C).

Feeding experiments were carried out in essentially three different ways. Germinating *Vinca* seeds were allowed to absorb water containing mevalonate-2-^{14}C (experiment 2, Table I) or 2-month old seedlings were fed mevalonate hydroponically (0.52% incorporation). With mature *Vinca* plants the cotton wick technique[6] was utilized (experiments 1 and 3, Table I).

Upon addition of 15 mg of carrier, loganic acid was isolated by the ion exchange method as previously described.[6] The crude acid was methylated and acetylated. The resulting loganin pentaacetate was recrystallized to constant activity and saponified back to loganin. The loganin was recrystallized to constant radioactivity and the data reveal relatively high rates of incorporation.

Emulsin hydrolysis[7] of loganin provides the aglucone, loganetin, and glucose. Determination of their respective specific activities established exclusive incorporation of mevalonate-2-^{14}C into the isoprenoid moiety (experiment 1, Table I). Decarboxylation studies

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(2) C. J. Coscia, L. Botta, and R. Guarnaccia, *Arch. Biochem. Biophys.*, **136**, 498 (1970).
(3) A. R. Battersby in "Natural Substances Formed Biologically from Mevalonic Acid," T. W. Goodwin, Ed., Academic Press, London, 1970, pp 157–168.
(4) In 1951 Jaminet[5] discovered a compound in *Strychnos nux vomica* which he identified as loganic acid on the basis of its chromatographic mobility, uv spectra, and appearance upon saponification of loganin.
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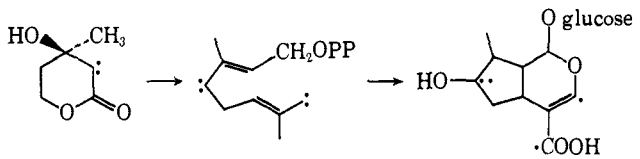
Table I. Degradation of Labeled Loganic Acid

Expt no.	% incorporation	Compound counted	Specific activity, μCi/mol	Relative molar activities
1[a]	1.16[c]	Loganic acid (as loganin)	360	1
		Aglucone	320	0.91
		Glucose	0	0
		$BaCO_3$ from C-11[e]	70	0.20
2[b]	0.36[c]	Loganic acid (as loganin)	51	1
		$BaCO_3$ from C-11	10	0.20
3[a]	0.01[d]	Loganic acid (as loganin)	38	1
		Aglucone	31	0.81
		Glucose	4.5	0.12
		CH_3COOH (from C-10 and C-8)	8.1	0.21

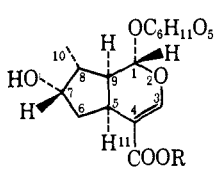
[a] Cotton wick. [b] Hydroponic. [c] Precursor: mevalonate-2-^{14}C. [d] Precursor: 3-hydroxy-3-methylglutaric acid-3′-^{14}C. [e] Two determinations.

on loganin indicated that 20% of the label in the aglucone is at C-11 as expected[3] (Table I, Scheme I).

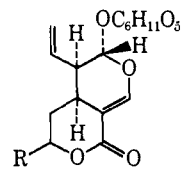
Scheme I



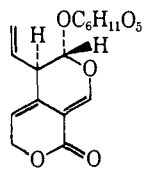
No difference in the extent of randomization of the terminal dimethyl groups was observed in *Vinca* plants at two different stages of development (experiments 1 and 2).



loganic acid, R = H
loganin, R = CH_3



sweroside, R = H
secologanic acid, R = OH



gentiopicroside

Degradation of 3-hydroxy-3-methylglutaric acid-3′-^{14}C labeled loganic acid revealed a considerable randomization of label (experiment 3). Hence the glucose contained 12% of the total radioactivity of loganic acid whereas carbons 8 and 10, isolated as acetic acid after Kuhn–Roth oxidation, possessed 21% of the total activity. Had the 3-hydroxy-3-methylglutaric acid been converted directly to mevalonate, C-10 should have possessed 50% of the label of the aglucone.

The biosynthesis of loganic acid from mevalonate in *V. rosea* plants at various stages of development establishes this compound as an active metabolite (Scheme I). Since loganin is a precursor of indole alkaloids,[3] preparation of cell-free extracts capable of converting loganic acid to loganin implicates the acid in the biogenesis of indole alkaloids.[8] That 7-desoxyloganin occurs in *V. rosea* and is also converted to indole alkaloids[3] suggests a dual pathway wherein methylation can occur at different points. Metabolic grids have been observed in the biosynthesis of plant metabolites including morphine, flavonoids, and the carotenoids.[9] Such a parallel pathway could also explain the incorporation of sweroside into indole alkaloids.[10] In this case oxidation to secologanic acid would be expected to precede methylation. Secologanic acid occurs in nature in a derivatized form as foliamenthin.[11] Alternately, methyl esters of the opened lactone of sweroside would not be expected to be sufficiently stable unless enzyme bound. In our hands the methyl ester of gentiopicroside was extremely labile rapidly reverting to gentiopicroside.

Results with 3-hydroxy-3-methylglutaric acid-3′-^{14}C as substrate indicate primarily breakdown to acetoacetate and acetate rather than a direct conversion to mevalonate. This is consistent with findings on isoprenoid synthesis from acetate fragments in mammalian and bacterial systems as well as in other plants; *i.e.*, free 3-hydroxy-3-methylglutaric acid is not an intermediate.[12]

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