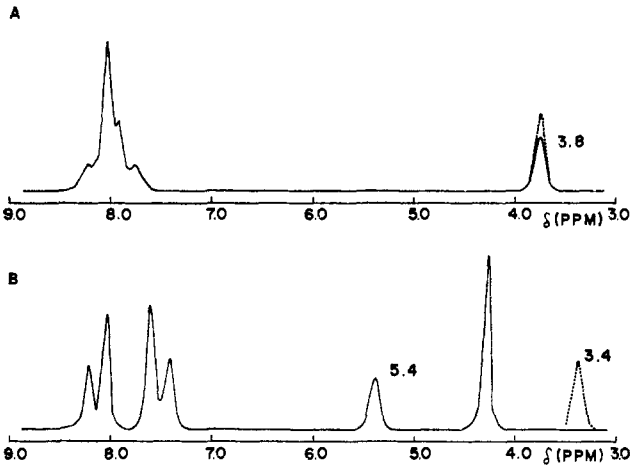


Figure 1. (A) Nmr spectrum of $^{+}C(Ph)_2CH_2CH_2C^{+}(Ph)_2$ and of $^{+}C(Ph)_2CH_3$. Both spectra are identical in the δ 8 ppm region (aromatic protons) but differ in their intensity in the δ 3.8 ppm region $-CH_2-C^{+}$ (or $^{+}C-CH_3$). Solid line corresponds to the ratio H (aromatic):H (aliphatic) 10:2 (dimer); the dotted line to the ratio 10:3. (B) Nmr spectrum of $^{+}C(C_6H_4OCH_3)_2CH_2Cl$ (II): δ 5.4 ppm, $>C^{+}-CH_2Cl$; δ 4.1 ppm, $-OCH_3$. The dimer, $^{+}C(C_6H_4OCH_3)_2CH_2CH_2C^{+}(C_6H_4OCH_3)_2$, or $^{+}C(C_6H_4OCH_3)_2CH_3$ do not show absorption at δ 5.4 ppm but at δ 3.4 ppm. Otherwise the spectrum is the same as that of II.

analysis and by its melting point. (2) A solution of $(CH_3OC_6H_4)_2C{=}CHCl$ reacts with CF_3COOH and gives colored species absorbing at λ_{max} 540 mμ with *no* shoulder at 500 mμ. (3) The nmr spectrum of $(CH_3OC_6H_4)_2C{=}CHCl$ in concentrated sulfuric acid is identical with that of II (although all the lines are slightly shifted because of the difference in the environment). No absorption is seen at δ 3.4 ppm.

The reaction of $SbCl_5$ with I is visualized as

$$SbCl_5 + (CH_3OC_6H_4)_2C{=}CH_2 \longrightarrow SbCl_4^- + \overset{+}{C}(C_6H_4OCH_3)_2CH_2Cl$$

i.e., as a transfer of Cl^+ from $SbCl_5$ to the olefin. Its detailed mechanism is now under investigation. The existence of $SbCl_4^-$ ions is demonstrated by the preparation of salts such as $(NH_4)^+ SbCl_4^-$ and its analogs.

The participation of free chlorine in the process is ruled out. The chlorine may be formed by the reaction $SbCl_5 \rightleftarrows SbCl_3 + Cl_2$; however, the reaction discussed above was not inhibited by the addition of $SbCl_3$ to $SbCl_5$ solution. In fact, a small excess of $SbCl_3$ was added to a solution of $SbCl_5$, and the mixture was chilled to $-70°$ after being kept at room temperature for several hours. On mixing it with a solution of I, cooled also to $-70°$, the conversion to $^{+}C(C_6H_4OCH_3)_2CH_2Cl$ took place in less than a second and the spectrophotometric analysis showed that the reaction was quantitative.

The hypothetical reaction

$$I + SbCl_5 \longrightarrow ClC(C_6H_4OCH_3)_2CH_2Cl + SbCl_3$$

followed by

$$SbCl_5 + ClC(C_6H_4OCH_3)_2CH_2Cl \longrightarrow SbCl_6^- + \overset{+}{C}(C_6H_4OCH_3)_2CH_2Cl$$

cannot be ruled out yet, although we believe it is improbable under our conditions. The problem may be solved by kinetic studies.

In the presence of an excess of I, the carbonium ion reacts as

$$ClCH_2\overset{+}{C}(C_6H_4OCH_3)_2 + I \longrightarrow ClCH_2C(C_6H_4OCH_3)_2CH_2\overset{+}{C}(C_6H_4OCH_3)_2$$

The latter carbonium ion absorbs most probably at 500 mμ.

Acknowledgment. The financial support of this investigation by the National Science Foundation and by the Petroleum Research Fund administered by the American Chemical Society is gratefully acknowledged.

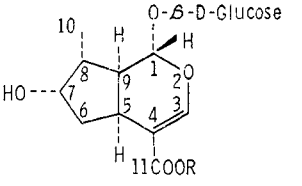
W. Bracke, W. J. Cheng, J. M. Pearson, M. Szwarc
Department of Chemistry, State University College of Forestry
Syracuse, New York 13210
Received September 27, 1968

Mechanism of Secoiridoid Monoterpene Biosynthesis

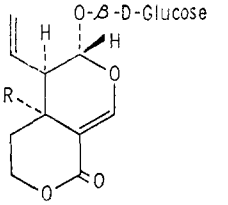
Sir:

In the biosynthesis of the nontryptamine moiety of indole alkaloids in *Vinca rosea*, both the intermediacy of loganin (**2**), an iridoid monoterpene glucoside, and the precursor relationship of sweroside (**3**), a secoiridoid monoterpene glucoside, have been demonstrated.[1-3] Since these glucosides have been found to be of mevalonoid origin, a general biogenetic scheme envisages their biosynthesis to proceed by the customary isoprenoid mechanism to geranyl pyrophosphate followed by eventual conversion of the iridoid monoterpene to the secoiridoid type.[4-8] We now have direct evidence to support both these hypotheses.

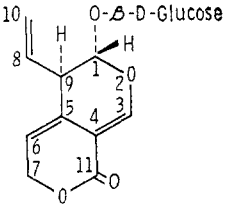
In the biosynthesis of all-*trans* isoprenoid compounds thus far examined, isomerization of isopentenyl pyro-



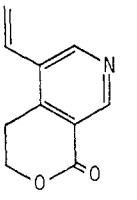
1 R = H
2 R = CH_3



3 R = H
4 R = OH



5



6

(1) A. R. Battersby, R. T. Brown, R. S. Kapil, J. A. Martin, and A. O. Plunkett, *Chem. Commun.*, 890 (1966).
(2) P. Loew and D. Arigoni, *ibid.*, 137 (1968).
(3) H. Inouye, S. Ueda, and Y. Takeda, *Tetrahedron Letters*, 3453 (1968).
(4) C. J. Coscia and R. Guarnaccia, *J. Am. Chem. Soc.*, **89**, 1280 (1967).
(5) H. Inouye, S. Ueda, and Y. Nakamura, *Tetrahedron Letters*, 3221 (1967).
(6) A. R. Battersby, R. S. Kapil, and R. Southgate, *Chem. Commun.*, 131 (1968).
(7) S. Brechbühler-Bader, C. J. Coscia, P. Loew, C. von Szczepanski, and D. Arigoni, *ibid.*, 136 (1968).
(8) C. J. Coscia and R. Guarnaccia, *ibid.*, 138 (1968).

Table I. Radioactivity of Monoterpene Derivatives and Their Degradation Products from *in vivo* Experiments on *Swertia caroliniensis*[a]

Experiment	Precursor	Monoterpene derivative	Specific act., dpm/mmole ^3H	Specific act., dpm/mmole ^{14}C	$^3H/^{14}C$
1	(4*R*)-Mevalonate				2.1
		Loganin pentaacetate	6.57×10^5	3.32×10^5	2.0
		Gentiopicroside tetraacetate	1.06×10^5	1.06×10^5	1.0
		Gentianine (**6**)	0	1.06×10^5	0
2	(4*R*)-Mevalonate				1.72
		Loganic acid pentaacetate	8.84×10^5	5.9×10^5	1.64
		Gentiopicroside tetraacetate	5.76×10^4	5.5×10^4	1.05
		Gentianine (**6**)	0	5.49×10^4	0
3	(4*S*)-Mevalonate				1.92
		Loganic acid pentaacetate	0	3.98×10^4	0
		Gentiopicroside tetraacetate	0	2.61×10^5	0
4	(4*S*)-Mevalonate				2.53
		Loganin pentaacetate	0	1.9×10^5	0
5	Loganic acid (**1**)		2.45×10^7	1.22×10^7	2.0
		Gentiopicroside tetraacetate	1.20×10^4	1.20×10^4	1.0
		Gentianine (**6**)	0	1.16×10^5	0

[a] Specific activities were determined by averaging values obtained from samples from two to three successive recrystallizations.

phosphate to dimethylallyl pyrophosphate and subsequent condensation of another isopentenyl pyrophosphate with the latter to form geranyl pyrophosphate entail elimination of the original *pro-S* hydrogen[9] and retention of the *pro-R* hydrogen on the C-4 methylene carbon of mevalonate.[11]

To examine this facet of the iridoid pathway, [2-^{14}C,(4*R*)4-^{3}H]mevalonate and [2-^{14}C,(4*S*)4-^{3}H]mevalonate were administered to young specimens of the higher plant, *Swertia caroliniensis*. In previous studies we have shown that this plant contains loganic acid (**1**) and the secoiridoid monoterpene glucoside, gentiopicroside (**5**).[4,8,12]

Feeding of mevalonate and isolation of **1** were carried out as previously described.[8] After dilution with authentic carrier **1** and conversion to either loganic acid pentaacetate or loganin pentaacetate, samples were recrystallized to constant radioactivity with changes in solvent of crystallization. Table I lists the actual specific activities measured.

The $^3H/^{14}C$ ratios of the loganic acid derivatives isolated from plants fed (4*R*)-mevalonate were found to be identical, within experimental error, with that of precursor (Table I). Alternatively (4*S*)-mevalonate afforded loganic acid bearing only a ^{14}C label, demonstrating that the elimination of hydrogen from C-4 of mevalonate in iridoid monoterpene synthesis entails the same stereospecificity as observed for higher terpenoids. Similar results have recently been obtained for loganin (**2**) in *Vinca rosea*.[14,15]

Gentiopicroside (**5**) a congener of loganic acid (**1**) in *Swertia*, was also isolated, converted to its crystalline tetraacetate, and recrystallized to constant radioactivity as previously described.[4] From the revised structure, **5**, of this amaroid it is apparent that one of the carbons derived from C-4 of mevalonate (C-5 in gentiopicroside) does not possess hydrogen, and it was expected that tritium labeling would be halved, *i.e.*, one tritium to two ^{14}C atoms. As seen in Table I, this was the case when (4*R*)-mevalonate was used as precursor. If (4*S*)-mevalonate was fed to the plant, the isolated gentiopicroside tetraacetate contained no tritium. Of the two isotopic hydrogens expected to be present in geraniol from (4*R*)-mevalonate, the C-5 proton must be eliminated after loganic acid is synthesized, while the C-9 hydrogen would appear to be retained in gentiopicroside. In the case of indole alkaloid biosynthesis, however, the C-5 proton is retained while C-9 is lost,[15] obviating the possible intermediacy of swertiamarin (**4**) or gentiopicroside (**5**) but not that of sweroside (**3**).[3]

Degradation of doubly labeled gentiopicroside tetraacetate to a tritium-less gentianine (**6**) (Table I, expt 1 and 2), as previously described,[4] affords evidence that the tritium is located at C-9. To obviate the possibility that tritium is present at C-6 and was labilized during the acid hydrolysis step of the conversion to **6**, inactive gentiopicroside tetraacetate was degraded to gentianine (**6**) in the presence of tritiated water. Only 10% tritium per mol of **6** was taken up under the conditions of hydrolysis.

From the *in vivo* experiment with (4*R*)-mevalonate (expt 1) a sample of doubly labeled loganic acid was isolated. Purification by silica gel chromatography (chloroform–methanol–water 4.5:4.5:1) was followed by repetitive preparative thin layer chromatography (silica gel H; chloroform–methanol 1:1). Radioactivity assays on successive runs indicated homogeneity, and the specific activity of the loganic acid precursor (expt 5) correlated well with that of a sample of **1** from the same plant, which was diluted, acetylated, methylated, and recrystallized to constant activity (expt 1). After administration of 30 mg of the labeled acid **1** to a young *Swertia* plant its gentiopicroside was isolated and recrystallized to constant radioactivity as its tetraacetate (1.3% incorporation). As indicated in expt 5, the gentiopicroside tetraacetate possessed the same isotope ratio as **5** which is derived from (4*R*)-mevalonate. Degradation to gentianine (**6**) (Table I, expt 5) again led to full

(9) See ref 10 for an explanation of the nomenclature.
(10) K. R. Hanson, *J. Am. Chem. Soc.*, **88**, 2731 (1966).
(11) G. Popjak and J. Cornforth, *Biochem. J.*, **101**, 553 (1966).
(12) In a recent communication[13] Professor Inouye and coworkers proposed a revised structure for gentiopicroside (**5**). Our labeling data are in agreement with this structure.
(13) H. Inouye, T. Yoshida, Y. Nakamura, and S. Tobita, *Tetrahedron Letters*, 4429 (1968).
(14) These workers have also established that, as in animals, the 3*R* enantiomer of mevalonate is the biological precursor in *Vinca rosea*.[15] On the assumption that this also holds for *Swertia*, it is the 3*R* stereoisomers of the racemic doubly labeled mevalonates which are utilized by the plant.
(15) A. R. Battersby, J. C. Byrne, R. S. Kapil, J. A. Martin, T. G. Payne, D. Arigoni, and P. Loew, *Chem. Commun.*, 951 (1968).

retention of ^{14}C but complete loss of tritium, thereby establishing the precursor relationship of loganic acid (**1**) to gentiopicroside (**5**).[16]

(16) Supported by National Science Foundation Grant GB 7411.
(17) Career Development Awardee of the National Institutes of Health.

Rocco Guarnaccia, Luigi Botta, Carmine J. Coscia[17]
Department of Biochemistry
St. Louis University School of Medicine, St. Louis, Missouri 63104
Received October 26, 1968

Biosynthesis of the Tetracyclines. XII.[1] Anhydrodemethylchlortetracycline from a Mutant of *Streptomyces aureofaciens*

Sir:

We recently reported the isolation of 4-aminodedimethylaminoanhydrodemethylchlortetracycline.[2] We commented at that time that no anhydrotetracycline derivative having the intact dimethylamino group had yet been observed as a product of a blocked mutant of *Streptomyces aureofaciens*. We have continued to search, however, for such a mutant, and now report a successful achievement of this goal. Anhydrodemethylchlortetracycline was successfully isolated from mutant 1E6113, a derivative of a demethylchlortetracycline-producing parental strain.[3] Mutant 1E6113 normally produces a small quantity of demethylchlortetracycline (DMCT) (about 10–25 μg/ml), but in the absorption spectrum of an acidic aqueous extract there was seen a significant amount of absorption in the 420–440 mμ region where the anhydrotetracyclines show a moderately strong maximum. Using the absorbancy in this region as a guide, we were able to isolate the substance responsible and to show that it was identical with an authentic specimen of anhydrodemethylchlortetracycline.[4] The isolation proceeded as follows: 1 l. of 1E6113 fermented mash (microbiological assay 25 μg/ml as DMCT) was acidified to pH 1.5 with perchloric acid, washed with hexane (centrifugation), and extracted with ethyl acetate (centrifugation). The extract, which by paper chromatographic examination contained essentially no demethylchlortetracycline, was concentrated to dryness and the residue, 840 mg, taken up in tetrahydrofuran (THF). The solution was dried onto 30 g of diatomaceous earth and packed onto the top of a 3 in. $\times$ 12 in. partition chromatographic column consisting of 300 g of diatomaceous earth filter aid which had been thoroughly mixed with 150 ml of 0.1 *M* ethylenediaminetetraacetic acid (EDTA) buffer at pH 8.3. The column was developed with chloroform and the eluate collected in 100-ml fractions. Spectrophotometric examination of the fractions showed anhydrotetracycline-like absorption in cuts 8 through 20. These were combined and evaporated to dryness to yield 80 mg of a partly crystalline product, identified as predominantly amphoteric anhydrodemethylchlortetracycline by paper chromatography (orange fluorescing spot at R_f 0.48 in pH 8.3 EDTA–butanol system). The compound, 80 mg, was converted to the hydrochloride by dissolving in 0.5 ml of 1-butanol plus 0.3 ml of 8 *N* hydrochloric acid. The crystalline hydrochloride, 53 mg, was shown to be pure anhydrodemethylchlortetracycline by exact correspondence of the ultraviolet and infrared spectra with those of an authentic specimen.[4] Paper chromatographic behavior, in two systems, of the isolated compound likewise was identical with that of the authentic specimen.

Although the original 1E6113 mash contained a small amount of demethylchlortetracycline, we took pains in the isolation to avoid conditions which might partly or completely dehydrate this compound to the anhydro derivative. In addition, the isolation method used was one which separated the already present anhydro derivative from the parent compound at an early point. Finally, the quantity of anhydrodemethylchlortetracycline isolated in pure form was greater than the total DMCT content of the starting mash. For these reasons we feel that there is no possibility that the anhydrodemethylchlortetracycline is an isolation artifact.

In that part of the biosynthetic pathway to the tetracyclines[5] which involves tetracyclic intermediates, blocked mutants have now been reported for each step, excepting only two. These two steps are: 4-hydroxylation of the pretetramids (a product of this hydroxylation, 4-hydroxy-6-methylpretetramid, is known[6]), and the 12a-hydroxylation of the 4-hydroxypretetramids to yield the 4-ketodedimethylaminoanhydrotetracyclines. Some experimental observations on the latter biosynthetic step will be presented in a forthcoming comunication.

(1) Previous paper in this series: J. R. D. McCormick, E. R. Jensen, N. H. Arnold, H. S. Corey, U. H. Joachim, S. Johnson, P. A. Miller, and N. O. Sjolander, *J. Am. Chem. Soc.*, **90**, 7127 (1968).
(2) J. R. D. McCormick, E. R. Jensen, S. Johnson, and N. O. Sjolander, *ibid.*, **90**, 2201 (1968).
(3) Mutant 1E6113 was isolated by Dr. J. Growich and Mr. N. Deduck of these laboratories.
(4) J. S. Webb, R. W. Broshard, D. B. Cosulich, W. J. Stein, and C. F. Wolf, *J. Am. Chem. Soc.*, **79**, 4563 (1957).
(5) J. R. D. McCormick in "Antibiotics," Vol. 2, D. Gottlieb and P. D. Shaw, Ed., Springer, New York, N. Y., 1967.
(6) J. R. D. McCormick, U. H. Joachim, E. R. Jensen, S. Johnson, and N. O. Sjolander, *J. Am. Chem. Soc.*, **87**, 1793 (1965).

J. R. D. McCormick, Elmer R. Jensen
Lederle Laboratories, American Cyanamid Company
Pearl River, New York
Received November 6, 1968

The Reaction of Complexes of Rhodium(I) Chloride with Norbornadiene

Sir:

Norbornadiene is oligomerized by several metal catalysts[1] to dimers and trimers that are related formally to the molecules of the starting hydrocarbon by a simple cycloaddition process.[1h,2,3] The contrasting hypotheses that the new carbon–carbon bonds form simultaneously[1i,4] or sequentially[2] have both been advanced. This communication reports the reaction of

(1) (a) R. Pettit, *J. Am. Chem. Soc.*, **81**, 1266 (1959); (b) C. W. Bird, R. C. Cookson, and J. Hudec, *Chem. Ind.* (London), 20 (1960); (c) C. W. Bird, D. L. Colinese, R. C. Cookson, J. Hudec, and R. O. Williams, *Tetrahedron Letters*, 373 (1961); (d) G. N. Schrauzer and S. Eichler, *Chem. Ber.*, **95**, 2764 (1962); (e) D. R. Arnold, D. J. Trecker, and E. B. Whipple, *J. Am. Chem. Soc.*, **87**, 2596 (1965); (f) P. W. Jolly, F. G. A. Stone, and K. Mackenzie, *J. Chem. Soc.*, 6416 (1965); (g) D. M. Lemal and K. S. Shim, *Tetrahedron Letters*, 368 (1961); (h) J. J. Mrowca and T. J. Katz, *J. Am. Chem. Soc.*, **88**, 4012, 5941 (1966); (i) G. N. Schrauzer, B. N. Bastian, and G. A. Fosselius, *ibid.*, **88**, 4890 (1966).
(2) T. J. Katz and N. Acton, *Tetrahedron Letters*, 2601 (1967).
(3) R. Hoffmann and R. B. Woodward, *J. Am, Chem. Soc.*, **87**, 2046 (1965).
(4) F. D. Mango and J. H. Schachtschneider, *ibid.*, **89**, 2484 (1967).