Relative molare Aktivitäten von Plumierid und seinen Abbauprodukten

Verbindung	Acetat-1-^{14}C-Experiment		Mevalonat-2-^{14}C-Experiment	
	Gef. %	Ber. %	Gef. %	Ber. %
Plumierid (I)	100,0	100	100,0	100
Hexahydro-desoxyplumierid (II)	99,9	100	98,7	100
Aglykon D (III)	99,8	100	100,4	100
Tetrahydro-desoxyplumierid (IV)	-	-	100,0	100
Tetrahydro-desoxyplumierid-Säure (V)	–	–	100,7	100
CO_2 aus der Carboxylgruppe von V[a]	–	–	24,9	25
Ameisensäure (VI)	0,45	0	25,1	25
Essigsäure (VII)[b]	45,2	50	0,85	0
Propionsäure (VIII)	44,6	50	1,1	0
Bernsteinsäure (IX)	4,1	0	44,3	50
Äthylbernsteinsäure (X)	90,3	100	0,91	0
CO_2 aus der Carboxylgruppe von VII[c]	41,3	50	–	–
Iodoform aus der Methylgruppe von VII[c]	1,1	0	–	–
CO_2 aus den Carboxylgruppen von X[d]	28,6	25	–	–

[a] Durch Hochvakuumpyrolyse von V, [b] durch modifizierte Kuhn-Roth-Oxydation, [c] durch Pyrolyse des Li-Salzes im Hochvakuum, [d] durch modifizierten Schmidtschen Abbau.

jenigen der aus zwei Mevalonsäure-2-^{14}C-Einheiten gebildeten terpenoiden Seitenkette im Mycelianamid[16]. Die Umwandlung Citronellal→Iridodial liess sich *in vitro* verwirklichen[17]. Die bisher nie beobachtete[15] Gleichverteilung der Markierung auf die C-Atome 3 und 15 im Plumierid erfordert die Einschaltung eines Zwischenproduktes, z.B. A, in dem diese zwei C-Atome einander vollkommen äquivalent sind.

Dasselbe Schema gilt vermutlich für die Plumericine und Fulvoplumierin. Ob das aus 10 Kohlenstoffatomen bestehende Aglykongerüst der in der Einleitung genannten terpenoiden Pflanzenstoffe ebenfalls aus zwei Mevalonsäureeinheiten aufgebaut wird, bleibt noch abzuklären. Soweit bekannt, entsprechen diese Verbindungen hinsichtlich ihrer absoluten Konfiguration dem Plumierid[18].

Summary. It has been shown that the leaves of *Pl. acutifolia* incorporate acetate-1-^{14}C and mevalonate-2-^{14}C specifically for the formation of the aglycone moiety of plumieride.

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Organisch-Chemisches Institut der Universität Zürich (Schweiz), 27. Februar 1964.

[16] A. J. BIRCH, M. KOCOR, N. SHEPPARD und J. WINTER, J. chem. Soc. *1962*, 1502.

[17] K. J. CLARK, G. I. FRAY, R. H. JAEGER und R. ROBINSON, Tetrahedron *6*, 217 (1959).

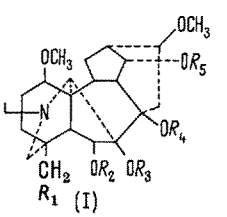
[18] Bekannt ist die absolute Konfiguration von Verbenalin[6], Genipin[4], Aucubin (siehe eine spätere Mitteilung) und damit auch von Catalposid (W. H. LUNN, D. W. EDWARD und J. T. EDWARD, Chem. and Ind. *1961*, 1488).

[19] Wir danken dem Schweizerischen Nationalfonds sehr für die Unterstützung der Arbeit, Herrn Dr. J. WÜRSCH (F. Hoffmann-La Roche & Cie., Basel) für markiertes Mevalonsäure-lakton und Herrn Prof. D. ARIGONI (ZÜRICH) für interessante Diskussionen.

The Biosynthesis of Diterpenoid Alkaloids

In recent years it has generally been assumed that the C_{20} and C_{19} skeletons of the alkaloids isolated from plants of the *Garraya*, *Delphinium* and *Aconitum* species were terpenoid, and as such produced, *in vivo*, from acetate units by the 'mevalonate pathway' so well established for the biosynthesis of many non-alkaloidal terpenes[1]. Thus these alkaloids are commonly referred to as the *diterpenoid alkaloids*.

The recent report by HERBERT and KIRBY[2] that there was no detectable incorporation of mevalonic acid-2-^{14}C into delpheline (I, R_1=H, R_2=H, R_3+R_4=CH_2, R_5=CH_3)[3] biosynthesized in *Delphinium elatum* is therefore most surprising and has prompted us to communicate the results of our initial experiments on the biosynthesis of brownine (I, R_1=OCH_3, R_2=CH_3, R_3=H, R_4=H, R_5=H)[4] and lycoctonine (I, R_1=OH, R_2=CH_3, R_3=H, R_4=H, R_5=CH_3)[5].



Sodium acetate-1-^{14}C, -2-^{14}C and mevalonic acid-2-^{14}C dibenzylethylenediamine salt were fed[6a] to *D. brownii* plants just prior to blossoming. After 8 days the above-ground portion of the plants were harvested, the alkaloids

Compound fed	Amount fed to 12 plants	% incorporation into alkaloids		Specific activity of pure alkaloids
		unhydrolysed	hydrolysed[a]	
Sodium acetate-1-^{14}C	250 μC	0.007	0.002	9.0×10^{-5} μC/mM
Sodium acetate-2-^{14}C	100 μC	0.006	0.003	2.5×10^{-4} μC/mM
Mevalonic acid-2-^{14}C, DBED salt	50 μC[b]	0.010	0.006	2.0×10^{-4} μC/mM

[a] Hydrolysis removes esterifying acids which, in the case of lycoctonine include methylsuccinic and anthranilic acids[5]. [b] Fed to 6 plants, 6 untreated plants were added prior to extraction of the alkaloids. All counting was performed using the Nuclear-Chicago 'Dynacon', Vibrating-reed electrometer-ionisation chamber assembly.

extracted, hydrolysed and separated by chromatography on alumina. Browniine was isolated as the crystalline perchlorate, lycoctonine as the crystalline hydrate.

From the results given in the Table it can be seen that although the 'incorporations' were poor[6b], they were consistent with the pattern expected from a 'normal' terpene biosynthesis. Within experimental error, the specific activities of the pure browniine and lycoctonine were the same, in all cases, consistent with a common precursor.

HERBERT and KIRBY suggested that the failure to incorporate mevalonic acid into the alkaloids of *D. elatum* was due to unsuccessful competition with the biosynthesis of non-alkaloidal terpenes. (They found 0.02% incorporation of mevalonic acid into β-sitosterol biosynthesized, over 4 days, in *D. elatum*.) However, we suspect that the primary site of alkaloid biosynthesis in *D. brownii* is in the roots and if this is also the case with *D. elatum*, the failure of HERBERT and KIRBY to observe incorporation of mevalonic acid into the alkaloids may be ascribed to their use of detached leaves for their experiments. Their further result, that methionine, methyl-^{14}C was incorporated (0.025%) into delpheline, primarily into the methoxyl groups of the alkaloid, under these conditions, implies either that there is metabolic equilibrium between methylated and demethylated alkaloids in the leaves, or more probably that methylation occurs in the leaves, of alkaloid biosynthesized elsewhere in the plant. There are certainly present in the leaves of *D. brownii* alkaloids which, from their chromatographic behaviour, appear to contain more free hydroxyls than browniine or lycoctonine and which we hope will prove to be the unmethylated precursors of these alkaloids.

Zusammenfassung. Die diterpenoiden Alkaloide Browniin und Lycoctonin wurden *in vivo* aus Mevalonsäure-2-^{14}C hergestellt. Sie zeigten, aus Acetat-1-^{14}C und -2-^{14}C gewonnen, eine geringere Wirksamkeit. Die Biosynthese dieser Alkaloide scheint somit der der anderen Terpene analog.

M. H. BENN and JUDITH MAY

Chemistry Department, University of Alberta, Calgary (Canada), January 31, 1964.

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[6] (a) Injected in aqueous solution into the stem cavities. (b) The incorporations could probably be improved by using the 'wick method' (E. LEETE, J. Amer. chem. Soc. *84*, 55 (1962)) and feeding earlier in the development of the plant.

A Procedure for the Preparation of Gram-quantities of Bacterial Cell Walls[1]

Many methods are available for the preparation of bacterial cell walls[2]. Most of these, however, suffer from the disadvantage that only small amounts of bacteria (usually to 400 mg dry weight) can be disrupted in a single operation. The use of commercially available blenders or homogenizers for the disruption of large quantities of microorganisms was originally described by LAMANNA and MALLETTE[3] but seems to have been overlooked by students of bacterial cell walls. We have found that the Omni-Mixer Highspeed Homogenizer[4] can be used for the efficient disruption of relatively large quantities of bacterial cells. This homogenizer offers the added advantage over other types of homogenizers in that its stainless steel mixing chamber is mounted on a movable bracket. The chamber can thus be lowered into a temperature-control bath. Using this homogenizer, together with a high-speed centrifuge with large capacity, gram-quantities of cell walls can be readily prepared.

Commercially available cells of *Micrococcus lysodeikticus*[5] were used in these experiments. The glass beads

[1] Publication No. 358 of the Robert W. Lovett Memorial Group for the Study of Crippling Diseases, Harvard Medical School at the Massachusetts General Hospital, Boston. This investigation has been supported by research grants from the Medical Foundation and the National Institute of Allergy and Infectious Diseases, National Institutes of Health, United States Public Health Service (Grant E-4282).
[2] M. R. J. SALTON, in *The Bacteria* (Eds. I. C. GUNSALUS and R. Y. STANIER, Academic Press, New York 1960), vol. I, p. 115.
[3] C. LAMANNA and M. F. MALLETTE, J. Bacteriol. *67*, 503 (1954).
[4] Ivan Sorvall, Inc., Norwalk (Connecticut).
[5] Miles Chemical Company, Division of Miles Laboratories, Inc., Elkhart (Indiana).