metabolic functions of a certain microorganism may not be a true representation of its chemical activity in nature. Particularly therefore there is a need of investigating the biochemical mechanisms whereby the metabolic processes of associative organisms are coupled together. On the basis of the findings described in this paper it may be concluded that the symbiotic technique as opposed to the classical pure culture method is useful in the study of the biochemistry of microbial associations existing in different natural conditions.

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Zusammenfassung

Die zusammenwirkenden, insbesondere symbiontischen Faktoren, die in einer Bakterienmischkultur zur Wirkung kommen, sind noch wenig bekannt. Der vorliegende Übersichtsartikel orientiert über die gegenseitige Förderung verschiedener Milchsäurebakterien in synthetischen Nährlösungen mit besonderer Berücksichtigung einiger biochemischer Faktoren. Bei allen Versuchen wurden bestimmte, für das Wachstum unentbehrliche Vitamine oder Aminosäuren aus den Nährlösungen weggelassen. Es ergab sich, dass verschiedene Milchsäurebakterienstämme in einer Nährlösung miteinander gedeihen können, in welcher der eine oder der andere Stamm für sich allein mangels lebenswichtiger Vitamine oder Aminosäuren nicht hätte gedeihen können. Einige Milchsäurebakterien sind somit zur Synthese der von anderen Organismen benötigten Vitamine und Aminosäuren befähigt. Beispiele: *Lactobacillus arabinosus 17–5* bildet die für *Streptococcus faecalis R* notwendige Folsäure (oder Folinsäure) und umgekehrt *Str. faecalis R* das für *Lb. arabinosus 17–5* unentbehrliche Phenylalanin (Abb. 1).

Auf Grund dieser symbiontischen Beziehungen zwischen verschiedenen Milchsäurebakterienstämmen wurde eine Methode, die sogenannte Symbiose-Technik, für die Untersuchungen der Biosynthese der verschiedenen Wachstumsfaktoren entwickelt. Die theoretische Grundlage dieser Methode ist vom Verfasser ausgearbeitet worden. Mit Hilfe dieser Methode wurden Untersuchungen über die Biosynthese von *p*-Aminobenzoesäure ausgeführt. Die Versuche zeigen, dass die Aminosäure Phenylalanin bei Milchsäurebakterien als Vitamin-Vorstufe auftritt.

Die Ergebnisse lassen vermuten, dass die erwähnten zusammenwirkenden symbiontischen Wachstumsfaktoren (Aminosäuren und Vitamine) bei der Entwicklung von Bakterienmischkulturen in der Natur eine wichtige Rolle spielen. Der Verfasser hebt besonders hervor, dass das Studium der Symbiose in der Natur mit Hilfe dieser neuesten Methode sehr interessant wäre. Es lässt sich denken, dass zum Beispiel bei diesem Vorgehen neue biochemische Prozesse und Wachstumsfaktoren gefunden werden, die mit Hilfe der klassischen Reinkulturmethoden nur schwierig festzustellen wären.

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Rauwolfia Alkaloids XXVII
The Conversion of 3-Isoreserpine to Reserpine

The synthesis of *d,l*-3-isoreserpine recently reported by WOODWARD[1] gives added import to the conversion of 3-isoreserpine to reserpine for which we wish to record two methods. The epimerization of reserpine and its derivatives at C-3 was previously stated to proceed to completion under the reaction conditions employed as far as could be measured[2]. This conclusion was based on the failure to isolate unepimerized reserpine from the reaction mixture. Mindful of the limitations of this means of assaying the completeness of the reaction, we applied the more precise method of paper chromatographic analysis to the problem. In the system benzene-cyclohexane (1:1) as the mobile phase on formamide-methanol (7:3) impregnated Whatman No. 1 paper, reserpine and 3-isoreserpine had R_f values of 0·32 and 0·82 respectively. This is a slight modification of the system already described for the paper chromatography of *Rauwolfia* alkaloids[3].

A study of the isomerization of reserpine in various new media led to the observation that in refluxing acetic acid a considerable amount of reserpine remains unepimerized. 3-Isoreserpine, prepared as already described[4]

[1] R. B. WOODWARD, Nichols Award Address, March 16, 1956. New York, N. Y.

[2] H. B. MACPHILLAMY, L. DORFMAN, C. F. HUEBNER, E. SCHLITTLER, and A. F. ST. ANDRÉ, J. Amer. chem. Soc. *77*, 1071 (1955). – H. B. MACPHILLAMY, C. F. HUEBNER, E. SCHLITTLER, A. F. ST. ANDRÉ, and P. R. ULSHAFER, J. Amer. chem. Soc. *77*, 4335 (1955).

[3] F. A. HOCHSTEIN, K. MURAI, and W. H. BOEGEMANN, J. Amer. chem. Soc. *77*, 3551 (1955). – A. F. ST. ANDRÉ, B. KORZUN, and F. WEINFELDT, J. org. Chem. *21*, in press (1956).

[4] H. B. MACPHILLAMY, C. F. HUEBNER, E. SCHLITTLER, A. F. ST. ANDRÉ, and P. R. ULSHAFER, J. Amer. chem. Soc. *77*, 4335 (1955).

by epimerization of reserpine in refluxing acetic anhydride, contained less than 0·01% reserpine as indicated by paper chromatography. Refluxing 3-isoreserpine obtained in this manner in acetic acid for 24 h gave an equilibrium mixture of the C-3 epimers from which a 15% yield of reserpine could be obtained. The separation of the two alkaloids from the acetic acid equilibrium mixture was easily accomplished by treatment with limited amounts of ethyl acetate at room temperature. 3-Isoreserpine is readily soluble while only moderate amounts of reserpine dissolve. An additional 5% of reserpine can be separated from the ethyl acetate soluble fraction by chromatography on a cellulose column impregnated with formamide using benzene-cyclohexane (1:1) as the eluting agent. Because of the stability of both reserpine and 3-isoreserpine in refluxing acetic acid, especially in the absence of oxygen, and the ease of separation of the two alkaloids, the unepimerized 3-isoreserpine can be recycled to enhance the overall conversion. The reserpine obtained by this process (m. p. 265–266°, $[\alpha]_D^{26°}$–118° (chloroform); *Analysis*, Calculated for $C_{33}H_{40}N_2O_9$: C 65·11%; H 6·62%; N 4·60%; Found: C 65·29%; H 6·75%; N 4·63%) showed an identical infrared absorption spectrum and identical pharmacological activity to natural reserpine.

A 3-isoreserpine derivative has been transformed into a compound of the reserpine series by a second method. We have already reported[5] that 3-isoreserpic acid forms an O-acetyl amino acid on treatment with acetic anhydride and pyridine at room temperature, indicating its reluctance to form a lactone. Reserpic acid lactone does not epimerize at C-3 and rings *C*, *D*, and *E* must thus be locked in the *trans-anti-cis* conformation. 3-Isoreserpic acid was therefore subjected to conditions favoring acid catalyzed isomerization and subsequent lactonization. The epimerization equilibrium can thereby be displaced in the desired direction. Treatment of isoreserpic acid hydrochloride (m.p. 277–279°C [dec.]; *Analysis*, Calculated for $C_{22}H_{28}O_5N_2 \cdot HCl \cdot \frac{1}{2}H_2O$; C59·24%; H6·78%; N6·28%; Found: C59·13%; H6·85%; N6·28%) obtained by potassium hydroxide-methanol hydrolysis of methyl isoreserpate (m.p. 220°C)[4] with refluxing acetic anhydride containing a small amount of acetic acid gave reserpic acid lactone (m.p. 310–314°C). The material was identified by comparison of melting point, mixed melting point and infrared spectrum with a sample of reserpic acid lactone.[6] Similarly this transformation could be effected in better yield in refluxing collidine containing *p*-toluenesulfonic acid and phosphorus pentoxide. Reserpic acid lactone has been converted previously to reserpine in these laboratories[6].

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Zusammenfassung

Es konnte gezeigt werden, dass das Gleichgewichtsgemisch Reserpin/3-Isoreserpin, das beim Kochen in Essigsäure erhalten wird, beträchtliche Mengen Reserpin enthält. Durch Abtrennung der beiden Komponenten dieses Systems ist die Umwandlung von 3-Isoreserpin in Reserpin ermöglicht.

3-Isoreserpsäure-hydrochlorid erfuhr in Acetanhydrid eine Isomerisation mit anschliessender Bildung des Lactons der Reserpsäure.

[5] C. F. HUEBNER, H. B. MACPHILLAMY, E. SCHLITTLER, and A. F. ST. ANDRÉ, Exper. *11*, 303 (1955).

[6] L. DORFMAN, A. FURLENMEIER, C. F. HUEBNER, R. LUCAS, H. B. MACPHILLAMY, J. M. MUELLER, E. SCHLITTLER, R. SCHWYZER, and A. F. ST. ANDRÉ, Helv. chim. Acta *37*, 59 (1953).

Rauwolfia Alkaloids XXVI. Stereochemistry at C-17

Reserpine and deserpidine have been formulated with the C-17 methoxyl *trans* to the groups at C-16 and C-18 (α-oriented in the absolute sense)[1], as best evidenced by the postulated neighboring group participation of the C-17 methoxyl in the internal quaternization of methyl reserpate tosylate[2]. This complex reaction occurs only under the comparatively rigorous conditions of refluxing collidine or dimethylformamide. In addition to the 30% yield of the inner quaternary salt and a smaller quantity of methyl anhydroreserpate, at least two other as yet unidentified crystalline products are formed. Therefore, corroboration for the assigned configuration of the C-17 methoxyl is desirable. This confirmation comes from two independent lines of evidence.

The previously reported product obtained from 3-*epi*-α-yohimbine in 70% yield by the action of *p*-toluenesulfonyl chloride in pyridine at 5°[3] has been reexamined and found to be the quaternary tosylate I. It is soluble in hot water, is neutral, shows the infrared absorption bands characteristic of the *p*-tosylate ion at 1168, 1118, 1029, and 1007 cm^{-1} [4], and lacks the band in the 2632–2500 cm^{-1} region indicative of a $>NH^{\oplus}$ group. Since compound I must almost certainly result from a concerted displacement of tosylate by N-4 with inversion at C-17, its ready formation under mild conditions and in good yield establishes the *trans* relationship of the groups at C-16 and C-17 in 3-*epi*-α-yohimbine. We have reported that deserpidinol yields α-yohimbyl alcohol on treatment with 48% hydrobromic acid by demethylation at C-17 and inversion at C-3[5]. Since under these conditions inversion at C-17 during the demethylation is very unlikely, the α-orientation of the C-17 methoxyl in deserpidine is also established.

The formation of internal quaternary ammonium salts during the tosylation of reserpinol and 3-*iso*reserpinol and the conversion to their crystalline iodides, II*a* (m.p. 345–350° (dec.)[6], $[\alpha]_D^{24°}$ + 122° (dimethylformamide); Analysis calculated for $C_{22}H_{29}N_2OI$: C 54·98%; H 6·10%; N 5·83%; Found: C 54·99%; H 6·30%;

[1] C. F. HUEBNER, H. B. MACPHILLAMY, E. SCHLITTLER, and A. F. ST. ANDRÉ, Exper. *11*, 303 (1955). – E. WENKERT and L. H. LIU, Exper. *11*, 302 (1955). – E. E. VAN TAMELEN and P. D. HANCE, J. Amer. chem. Soc. *77*, 4692 (1955).

[2] E. WENKERT and L. H. LIU, Exper. *11*, 302 (1955). – E. E. VAN TAMELEN and P. D. HANCE, J. Amer. chem. Soc. *77*, 4692 (1955).

[3] F. E. BADER, D. F. DICKEL, C. F. HUEBNER, R. A. LUCAS, and E. SCHLITTLER, J. Amer. chem Soc. *77*, 3547 (1955).

[4] F. L. WEISENBORN and D. BURN, J. Amer. chem. Soc. *75*, 259 (1953).

[5] H. B. MACPHILLAMY, C. F. HUEBNER, E. SCHLITTLER, A. F. ST. ANDRÉ, and P. R. ULSHAFER, J. Amer. chem. Soc. *77*, 4335 (1955).

[6] Erroneously reported as m.p. 315–316° (dec.) in paper C. F. HUEBNER and E. WENKERT, J. Amer. chem. Soc. *77*, 4180 (1955).