enzymes have been adapted to a closely analogous functional behavior. Intermediates with critically tuned chemical properties may be essential for the efficiency of enzyme catalysis, and this close functional similarity might be the result of an intense selection pressure exerted on these two proteins during biological evolution.

The results with aspartate aminotransferase provide direct experimental indication of a possible mechanism for a multistep enzyme catalysis. The syncatalytic activation of a particular functional residue, produced by catalysis-linked conformational alterations of the enzyme-coenzyme-substrate complex, might well reflect the generation of a transient active site topochemistry optimally adapted for stabilization of an intermediate and/or catalysis of the neighboring steps in a multistep enzymatic reaction. Thus, the triad: conformational adaptability, syncatalytic changes in reactivity of functional groups, multistage reaction, might be interdependent features of enzyme catalysis[49, 50].

Zusammenfassung

Die Untersuchung von Enzymsubstratkomplexen auf katalyse-induzierte Änderungen der chemischen Reaktivität hat sich als ein aufschlussreicher experimenteller Zugang zum Mechanismus enzymatischer Reaktionen erwiesen. Ternäre Systeme bestehend aus Enzym, Substrat und einem geeigneten Reagens (Tetranitromethan = TNM) sind auf katalyse-abhängige Reaktionen geprüft worden, die nur im vollständigen System, jedoch nicht mit Reagens und Enzym allein oder Reagens und Substrat allein beobachtet werden. Untersuchungen mit Aldolasen und Aspartat-Aminotransferase haben ergeben, dass katalyse-induzierte Reaktivität sowohl auf dem Substrat- als auch auf dem Enzymteil eines Enzym-Substratkomplexes nachgewiesen werden kann.

Im Reaktionsmechanismus von Muskelaldolase (eine Lysinaldolase) und von Hefealdolase (eine Metallaldolase) lässt sich mit TNM ein intermediäres Carbanion des Substrats nachweisen. Die TNM-carbanion-Reaktion lässt sich spektralphotometrisch verfolgen und ist benutzt worden, um carboxypeptidase-behandelte Muskelaldolase und den Effekt von Cofaktoren auf die Hefealdolase zu untersuchen.

Katalyse-induzierte Reaktivitätsveränderungen einer Enzymseitenkette sind bei der Aspartat-Aminotransferase beobachtet worden. Ein essentieller Tyrosylrest, der in der Abwesenheit von Substrat nicht mit TNM reagiert, wird ungewöhnlich reaktiv im Laufe der Katalyse und ermöglicht dabei seine selektive Nitrierung.

Die katalyse-synchrone oder synkatalytische Aktivierung dieses Aminosäurerests scheint ein integraler Bestandteil des katalytischen Mechanismus von Aspartat-Aminotransferase zu sein und wird vermutlich durch katalyse-induzierte Konformationsänderungen des Enzym-Coenzym-Substratkomplexes hervorgebracht. Mögliche funktionelle Zusammenhänge synkatalytischer Reaktivitätsänderungen funktioneller Gruppen mit der Konformationsflexibilität des Enzymproteins und dem Vorkommen metastabiler Zwischenprodukte in der Enzymkatalyse werden diskutiert.

[49] Acknowledgements. I am grateful to J. F. Riordan for his collaboration throughout this work. I would also like to thank Prof. B. L. Vallee for his advice and encouragement and J. H. Kägi for helpful discussions. The excellent technical assistance of Charlotte Hart is gratefully acknowledged.

[50] This work was supported by Grant-in-Aid No. GM-15003 from the National Institutes of Health of the Department of Health, Education, and Welfare. The author received stipends from the Stiftung für Biologisch-Medizinische Stipendien der Schweizerischen Akademie der Medizinischen Wissenschaften (1967–68), the University of Zürich and the American-Swiss Foundation for Scientific Exchange (1969).

SPECIALIA

Bazzanenol, a New Sesquiterpene Alcohol Having a Skeleton of Bicyclo[5.3.1] Undecane System from Hepaticae, *Bazzania pompeana* (Lac.) Mitt.[1]

In a previous paper[2], it was reported that a sesquiterpene hydrocarbon of a new carbon skeleton, bazzanene, was isolated from an essential oil of *Bazzania pompeana* (Lac.) Mitt. (Japanese name Ômukadekoke) belonged to Lepidoziaceae, and its structure was determined as formula (I).

From the same essential oil, subsequently, a new sesquiterpene alcohol was isolated and its structure was determined as 2, 6, 6-trimethyl-8-methylene bicyclo[5.3.1]-undec-2-en-4-ol (II). For this alcohol we propose the name bazzanenol, and here present the evidence for the structural assignment.

This alcohol, $[\alpha]_D + 19.1°$, was isolated in a homogeneous state with respect to gas chromatography by means of the combination of fractional distillation and elution chromatography (silica gel and n-hexane involving 15% ethyl acetate). It has a molecular formula of $C_{15}H_{24}O$ upon the determination of the molecular ion (m/e calcd. 220.183, obsd. 220.184), and exhibited IR-absorption bands (liquid film) and NMR-signals (in $CDCl_3$) attributable to a hydroxyl group and a proton on the carbon atom bearing a hydroxyl group respectively at 3300 and 1019 cm^{-1}, 1.56 ppm (1H, exchangeable with D_2O) and 4.15 ppm (1H, m). When the compound was treated overnight with acetic anhydride in pyridine at room temperature, it easily produced an acetylated product which showed characteristic signals at 2.08 ppm (3H, s, $-OCOCH_3$) and 5.40 ppm (1H, m, H–C(–OAc)–). On the basis of these evidences the compound was characterized as a secondary alcohol.

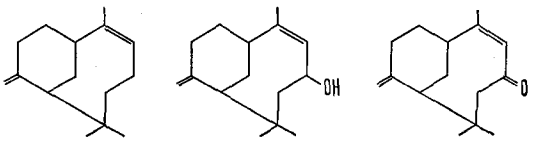
NMR- and IR-spectra of this alcohol also indicate the presence of an exomethylene group (1645 and 888 cm^{-1}, 4.87 and 5.06 ppm, each 1H and s), a partial structure of CH_3–C(=)–C(=)–H (823 cm^{-1}, 1.80 ppm, 3H, d, J = 1.5 Hz, 5.49 ppm, 1H, broad d, J = 5.0 Hz) and one geminal dimethyl group (1382 and 1377 cm^{-1}, 0.90 and 1.06 ppm, each 3H and s). All these groups were recognized in the previously reported hydrocarbon, bazzanene, and the whole pattern of the NMR-spectrum, excepting signals related to the hydroxyl group, resembles closely the spectrum of bazzanene, although 2 signals of the vinyl proton and of the methyl group attached to the double bond are both split into doublets in this alcohol. Such spectrometric resemblance between bazzanene and bazzanenol, and the above fact that the vinyl proton signal is split into doublet, make us assume bazzanenol to be a secondary alcohol which has a hydroxyl group at C_4-position of bazzanene.

In order to give the positive proof for this assumption, the alcohol was oxidized with Jones reagent, and an α,β-unsaturated ketone, $C_{15}H_{22}O$ (m/e calcd. 218.167, obsd. 218.166, ν_{max}^{liq} 1672 cm^{-1}, $\delta_{ppm}^{CDCl_3}$ 6.50, 1H, m) was obtained. These IR-, NMR- and mass-spectra of this ketone coincided respectively with those of a ketone (III) derived from bazzanene by sodium dichromate oxidation[2].

Zusammenfassung. Isolierung und Strukturaufklärung eines neuen Sesquiterpenalkohols aus *Bazzania pompeana* (Lac.) Mitt.

S. HAYASHI and A. MATSUO

Department of Chemistry,
Faculty of Science, Hiroshima University,
Hiroshima (Japan), 28 October 1969.



(I) (II) (III)

[1] Studies on chemical constituents from Hepaticae, Part III: S. HAYASHI, A. MATSUO and T. MATSUURA, Tetrahedron Letters, *1969*, 1599 and ref. [2] are regarded as Part I and II.

[2] S. HAYASHI, A. MATSUO and T. MATSUURA, Experientia *25*, 1139 (1969).

The Biosynthesis of Trisporic Acids from β-Carotene via Retinal and Trisporol

The trisporic acids, obtained from 'mated' cultures of *Blakeslea trispora*, include the 9-*cis* and -*trans* isomers[1] of trisporic acid C (Ia)[2], the main component, and of the corresponding 13-keto-compound trisporic acid B (Ib). The acids promote carotene and steroid synthesis in (–)-*B. trispora*[3], apparently by an enzyme-induction mechanism[4]. Moreover the 'gamones' which, in heterothallic Mucorales generally, diffuse between opposite mating types and elicit sexual differentiation, have been identified with trisporic acids[5]. In particular, the active extract from mated *Mucor mucedo* has afforded identifiable *cis*- and *trans*-trisporic acids A, B, and (principally) C, and conversely pure methyl trisporate-B and trisporate-C have activity towards (–)-*M. mucedo* which is quantitatively comparable to that of the total extract from mated strains of the same fungus[6].

Amongst the many co-metabolites of (I) which are peculiar to mated cultures of *B. trispora* is a series of neutral compounds, of which in our experience the major component is a substance which we have now characterized and designate as trisporol-C, (II). Isolated chromatographically, it has the molecular formula $C_{18}H_{28}O_3$ (from high resolution mass spectrometry); it has the characteristic trienone chromophore as seen in (I) and the fragmentation pattern in the mass spectrometer indicates the structure (II). This structure was confirmed by a partial synthesis from (Ia) by $LiAlH_4$ reduction of the corresponding ketal; compared with the natural material, the product had identical UV-, IR- and mass-spectra and was chromatographically indistinguishable on silica gel plates with a range of solvents and colour sprays. Since in the precursor-incorporation experiment reported below, the dilution of radioactivity in (II) was markedly less than in (Ia), we believe that this primary alcohol is a near precursor of the trisporic acids. Its physiological role is under investigation.

Earlier, by using 2-^{14}C-mevalonate we had shown that (I) contains the remains of 4 isoprene units, and adduced arguments that the monocyclic C_{18} skeleton of the trisporic acids arises from β-carotene[7]. In particular, when ^{14}C-β-carotene (labelled biosynthetically from 2-^{14}C-acetate)

[1] Upjohn Co., Netherlands Patent No. 6,512,313 (1966).

[2] L. CAGLIOTTI, G. CAINELLI, B. CAMERINO, R. MONDELLI, A. PRIETO, A. QUILICO, T. SALVADORI and A. SELVA, Tetrahedron (Supplement) *7*, 175 (1966).

[3] D. M. THOMAS and T. W. GOODWIN, Phytochem. *6*, 355 (1967).

[4] D. M. THOMAS, R. C. HARRIS, J. T. O. KIRK and T. W. GOODWIN, Phytochem. *6*, 361 (1967).

[5] H. VAN DEN ENDE, J. Bact. *96*, 1298 (1968).

[6] D. J. AUSTIN, J. D. BU'LOCK and G. W. GOODAY, Nature *223*, 1178 (1969).

[7] D. J. AUSTIN, J. D. BU'LOCK and D. J. WINSTANLEY, Biochem. J. *113*, 34P (1969).