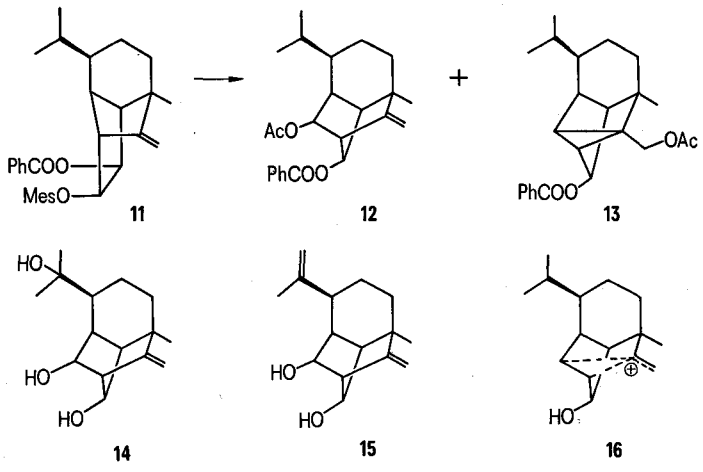
ableitenden Epoxids **8**. Dieses geht beim kurzen Erhitzen auf 295° in den Allylalkohol **9** über, dessen Vinylaethergruppierung im IR-Spektrum zu einer scharfen Bande bei 1655 cm^{-1} Anlass gibt. Die Umgebung dieser Gruppierung und ihre relative Lage zur allylischen Hydroxylfunktion folgt eindeutig aus NMR-Entkopplungsexperimenten. Die Bildung von **9** dürfte durch Wasserabspaltung aus der internen Halbacetalform einer aldehydischen Zwischenstufe erfolgen, welche in einem Retro-Prins ähnlichen Prozess aus **8** (siehe Pfeile) hervorgehen kann.

Beweisend für Struktur **4** ist die Partialsynthese der Substanz aus dem Sativendiol **1**. Das daraus über das Monobenzoat erhältliche Mesylat **11** ergibt durch Acetolyse bei 65° ein 1:3-Gemisch von **12** und **13**. Das Nebenprodukt ist auch durch Acetylierung von **5** zugänglich und lässt sich durch alkalische Hydrolyse in **4** überführen. Die dem Hauptprodukt der Reaktion zugeordnete Struktur **13** fusst im wesentlichen auf NMR-Daten und mechanistischen Überlegungen.

Für einen weiteren Pilzmetaboliten, $C_{15}H_{24}O_3$, Smp. 206°, $[\alpha]_D^{25} = +11°$ (EtOH), konnte Struktur **14** durch



eine direkte Verknüpfung ermittelt werden. Behandlung des Diacetats von **14** mit Phosphoroxytrichlorid in Pyridin und anschliessende Verseifung liefern **15** nebst dem Isopropylidenisomeren. Unter gleichen Reduktionsbedingungen ergeben sowohl **4** wie auch **15** ein in jeder Hinsicht identisches 4:1 Gemisch zweier epimerer gesättigter Derivate mit endo- bzw. exo-Lage der sekundären Methylgruppe.

Der den beiden neuen Metaboliten **4** und **14** zugrunde liegende Kohlenwasserstoff Isosativen ist zwar chemisch aus Sativen unter aequilibrierenden Bedingungen hergestellt worden[5], konnte aber bisher weder als solcher noch in Form von Derivaten aus natürlicher Quelle isoliert werden. Das hier nachgewiesene gleichzeitige Vorkommen des *cis*-1,2-Diols **1** und des *cis*-1,3-Diols **4** lässt vermuten, dass ihre Bildung über die gemeinsame kationische Zwischenstufe **16** erfolgt.

Summary. The structure of two new sesquiterpene metabolites of *Helminthosporium sativum*, previously assigned to the sativene series, has been settled as in **4** and **14** by chemical correlation.

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[5] L. SMEDMAN und E. ZAVARIN, Tetrahedron Lett. *1968*, 3833. – J. E. MCMURRY, J. org. Chem. *36*, 2826 (1971).

[6] Wir danken Sandoz AG (Basel) für finanzielle Unterstützung und Dr. E. HAERRI für Mithilfe bei der mikrobiologischen Arbeit.

The Synthesis of a Peptide Having the Structure Attributed to a Sound Habituating Material

A substance produced in brain concomitantly with a habituation to a bell sound stimulus has been recently isolated from trained rats. The active material was shown to be a peptide and named 'ameletin'. On the basis of microdansylation and a chymotrypsin digestion, the compound contained 6 amino acids – Ala, Glu, Gly, Lys, Ser, Tyr – and a Tyr-Ser linkage. A pyroglutamic acid residue was inferred from a negative ninhydrin test, while the C-terminal was identified as lysine[1]. Using the above information, plus the characterization of 3 dipeptides produced by hydrolysis with dipeptidyl aminopeptidase, the structure L-Glu-Ala-Gly-Tyr-Ser-Lys-OH was assigned to ameletin[2,3]. We now wish to report a preparation of this hexapeptide, as well as the results of the biological and chromatographic comparisons, which were done in the summer of 1973.

The protected amino acid (I)[4] was converted into the amine (II), which was joined to the hydrazide (III)[5] by the azide procedure. Tripeptide (IV), mp 113°, was

[1] G. UNGAR and S. R. BURZYNSKI, Fedn. Proc. *32*, 367 (1973), abstract 844.

[2] G. UNGAR, personal communication (May 6, 1973).

[3] G. UNGAR, Life Sci. *14*, 595 (1974). - Biochem. Pharmac. *23*, 1553 (1974).

[4] E. WÜNSCH and A. TRINKL, Hoppe-Seyler's Z. physiol. Chem. *345*, 193 (1966).

[5] A. A. COSTOPANAGIOTIS, B. O. HANDFORD and B. WEINSTEIN, J. org. Chem. *33*, 1261 (1968).

Thin-layer chromatography data

Solvent system	Natural[a]	Synthetic[b]	Synthetic[c]
n-BuOH/EtOH/HOAc/H_2O (8:2:1:3)	~0.4	0.38	0.20
Pyr/MEK/HOAc/H_2O (15:70:2:15)	~0.1–0.2	0.20	0.12
EtOH/Pyr/HOAc/EtOAc/H_2O (5:1:2:2:2)	~0.5	0.47	0.54
n-BuOH/Pyr/HOAc/H_2O (15:10:3:24)	—	—	0.28

[a] Determined in Houston by transfer bioassay. [b] Determined in Houston, silica gel sheets (K301R2). [c] Determined in Chicago-Seattle, silica gel G activated plates. In order to locate any impurities, 0.5 mg was applied; detection was by UV-fluorescence, ninhydrin, iodine, and choline-toluidine methods. Only one spot was seen in all solvent systems.

deblocked to give the oily amine (V), and a coupling with the blocked dipeptide (VI)[6], mp 132°, via the activated ester (VII), mp 154°, produced pentapeptide (VIII), mp 160°. The amine (IX) was reacted with the active ester (X)[7] to yield the hexapeptide (XI), mp 193°. Hydrogenolysis formed the amine (XII) and treatment

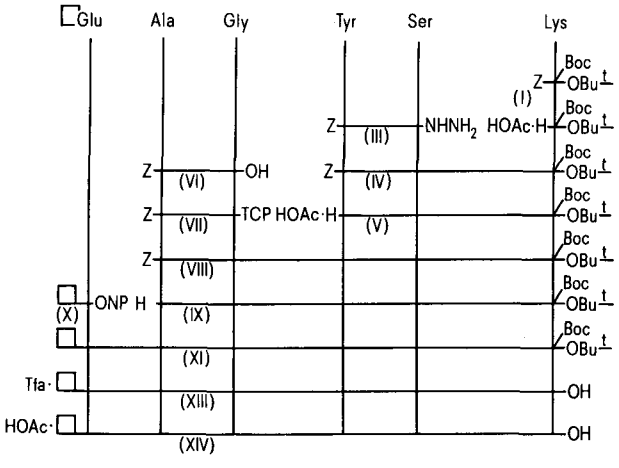

Fig. 1. Synthetic route to the hexapeptide.

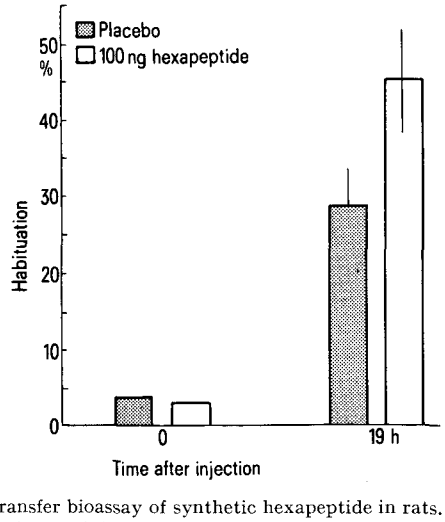

Fig. 2. Transfer bioassay of synthetic hexapeptide in rats. Male rats (220–250 g) were injected i.p. with 50 µl of vehicle or 100.0 ng of peptide. Just after injection and 19 h thereafter the number of startle responses per 100 sequential stimulations were charted using the same type bell and conditions as in the original assay of crude brain extracts[9]. The increase in speed of habituation as a consequence of injection of the hexapeptide is significant to $p = 0.001$ at 19 h.

with a solution of trifluoroacetic acid-anisole furnished L-pyroglutamyl-L-alanylglycyl-L-tyrosyl-L-seryl-L-lysine trifluoroacetate (XIII), mp 211°. Passage of XIII through an ion-exchange resin column (AG-1-X2) generated the equivalent acetate salt (XIV), which, after lyophilization, was left as a white powder, mp 173°. Peptide XIV had a satisfactory amino acid analysis (Ala, 0.98; Glu, 0.95; Gly, 1.00, Lys, 0.97; Ser, 0.80; Tyr, 0.96). On paper electrophoresis (pH 3.5, 1000 V, 2 h) the compound appeared at 2.2 cm vs cathode as a single pinkish-red ninhydrin positive spot; under the same conditions leucine was seen at 2.4 cm. This preparation is summarized in Figure 1.

A tabulation of the Rf constants for the natural material[8] and our synthetic compound is given in the Table. It will be noted that the values obtained in Houston support a close, but not identical nature for the 2 substances. By contrast, somewhat different numbers were seen in Chicago-Seattle for the authentic hexapeptide, and the reason for this discrepancy may be due to use of different media or other factors.

The comparison between natural rat ameletin and our synthetic hexapeptide was made in Houston by a transfer method using naive mice as recipients. That is, different doses of peptide were injected and a level was identified that gave the best reduction in habituation time. This bioassay technique is the procedure described in the original sound habituation test[9]. Fairly pure rat ameletin had maximum activity at 500 ng/mouse, while a sample of the synthetic peptide possessed habituation activity in the range of 50–200 ng/mouse[10].

In order to establish that a synthetic product is identical with a natural product, both compounds should have identical physical and chemical constants. Moreover, a synthetic material must show biological activity in the species from which the natural material is itself isolated. As can be seen from the sound habituation data presented in Figure 2, a physiologically reasonable dose of the hexapeptide does indeed improve bell sound habituation in transfer bioassays using recipient rats[11]. Unfortunately, the scarcity of natural ameletin now and in the near future rules out further detailed comparisons with the synthetic sample.

[6] A. Ali, R. M. Cook and B. Weinstein, Int. J. Prot. Res. *4*, 177 (1972).
[7] H. Gibian and E. Klieger, Justus Liebigs Annln Chem. *640*, 145 (1961).
[8] G. Ungar, personal communication (July 16, 1973).
[9] G. Ungar and C. Oceguera-Navarro, Nature *207*, 301 (1965).
[10] G. Ungar, personal communication (December 3, 1973).
[11] The preparation and biological assay of the synthetic hexapeptide have been briefly described: B. Weinstein and H. N. Guttman, Trans. Am. Soc. Neurochem. *5*, 173 (1974).

From the above experiments, it is clear that the proposed primary sequence may be close to the natural product. However, other alternatives, such as a cyclic formulation or even N-acetyl and N-formyl derivatives, can fit the known chemical data. Note that a molecular weight measurement on the original substance is still necessary in order to support a possible monomeric cyclic peptide arrangement (for which several possibilities can be written)[12]. Whether these and other compounds will yield the same enzymatic cleavage fragments, much less the biological activity of the natural product, is uncertain at this time[13,14].

Summary. A peptide isolated from rats habituated to a sound stimulus has been given the structure L-Glu-Ala-Gly-Tyr-Ser-Lys-OH. A synthesis of this compound afforded a product that different from the natural material on the basis of chromatographic and physiological comparisons. The proposed sequence must therefore be in error.

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[12] Since the free N^{ε}-amino group in lysine will routinely give a positive ninhydrin reaction, then the natural peptide must possess blocking groups at both the glutamic acid and lysine residues.

[13] After this work was completed, a second synthesis appeared: H. LACKNER and N. TIEMANN, Naturwissenschaften *61*, 217 (1974). Although the product possessed a broader mp of 170–177°, the chromatographic properties were said to be identical with our peptide. Unfortunately, a direct comparison was not possible between the Seattle and Göttingen peptides, due to a lack of additional material from the latter group. In any event, little biological activity was found and it was concluded that a mistake existed in the original structural investigation.

[14] G. UNGAR, personal communication (June 5, 1975). The possible presence of some cofactor has been suggested as an explanation for the higher activity of the natural product.

Occurrence of 4-Hydroxyphenylpyruvic Acid Oxime in the Marine Sponge *Hymeniacidon sanguinea*

Sponges of the family Verongidae provide a series of closely related compounds which may be considered as metabolites of 3,5-dibromotyrosine, including aerothionin (**1**)[1], homoaerothionin (**2**)[1] and the nitrile aeroplysinin-1 (**3**)[2].

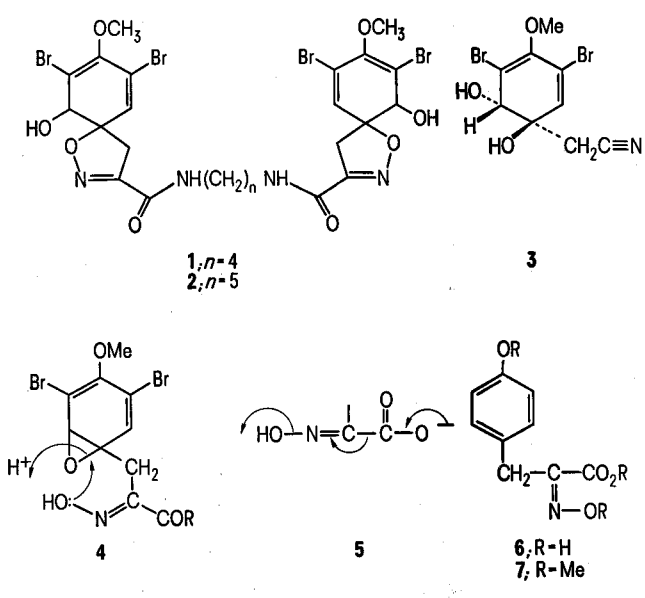
The spiro system in **1** and **2** could arise in various ways, including nucleofilic attack by an oxime function on an arene oxide as shown in **4**. Following certain suggestions that nitriles may be derived in vivo from α-amino-acids by way of α-keto- and α-oximino-acids[3], it has been speculated that the oxime **4** could be also a likely precursor of the nitrile aeroplysinin-1, as indicated in **5**[1].

We now have good support for the hypothesis of an oxime precursor of these compounds, by isolating from a marine sponge, *Hymeniacidon sanguinea*, the oximino-pyruvic acid **6**.

Fresh sponge (50 g, dry weight after extraction), collected near Roscoff[4] (France), was extracted (×3) with cold acetone for 3 days; solvent was removed and the aqueous residue was extracted with ether and *n*-butanol. After evaporation, 2 g *n*-butanol soluble material was dissolved in H_2O and applied to a column (2×20 cm) of Dowex 50W-X2, H^+ form. After washing with 1 *N* HCl, the crude 4-hydroxyphenyl-pyruvic acid oxime (**6**, 0.5 g) was eluted with H_2O. The NMR-spectrum (deuteriated acetone) showed 2 broad doublets (J 8Hz) centered at δ 6.7 and 7.2 for the aromatic protons consistent with a 1,4-disubstituted benzene system and a b singlet at δ 3.8. The UV, λ_{max} 279 nm (MeOH) bathochromically shifted by addition of alkali to 290 nm, was indicative for a phenol structure.

Further purification[5] was carried out on the permethyl-derivative, prepared with CH_2N_2 (in methanol, 1 h at r.t.) or with methyl iodide and silver oxide in chlorophorm at r.t. PLC on silica gel (Merck F_{254}; eluent: benzene) of the product gave **7** (in ca. 50% yield based on the crude material), as oil, M^+/e 237; λ_{max}^{MeOH} 225 and 275 nm (ε, 9,900; 2,370); $\nu_{max}^{liquid\ film}$ 1725, 1610 and 1510, 1040, 840 and 815 cm^{-1}; δ (100 MHz, CCl_4, ppm from TMS), 7.04 (2H, d, J 8 Hz), 6.65 (2H, d, J 8 Hz), 4.00 (3H, s, OCH_3) and overlapping 2 sharp singlets centered 3.75 for 2 OCH_3 and the benzylic CH_2.

The structure of 3-(4-hydroxyphenyl)-2-oximinopropionic acid for this sponge metabolite was definitively proved by converting **6** to tyrosine, and synthesizing the methyl 3-(4-methoxyphenyl)-2-methoximino-propionate (**7**).



[1] K. MOODY, R. H. THOMSON, E. FATTORUSSO, L. MINALE and G. SODANO, J. chem. Soc. Perkin *1*, 18 (1972).

[2] E. FATTORUSSO, L. MINALE and G. SODANO, J. chem. Soc. Perkin *1*, 16 (1972); L. MAZZARELLA and R. PULITI. Gazz. Chim. ital. *102*, 391 (1972).

[3] B. B. STOWE, Fortschr. Chem. org. Nat. Stoffe *17*, 248 (1959). A. AHMAD and I. D. SPENCER, Can. J. Chem. *38*, 1625 (1960).

[4] We are grateful to the Station Biologique de Roscoff (Nord-Finistère, France) for their hospitality which enabled us to collect the sponge.

[5] Difficulty was experienced in purification of the natural compound because its facile conversion to the *p*-hydroxyphenylacetic acid.