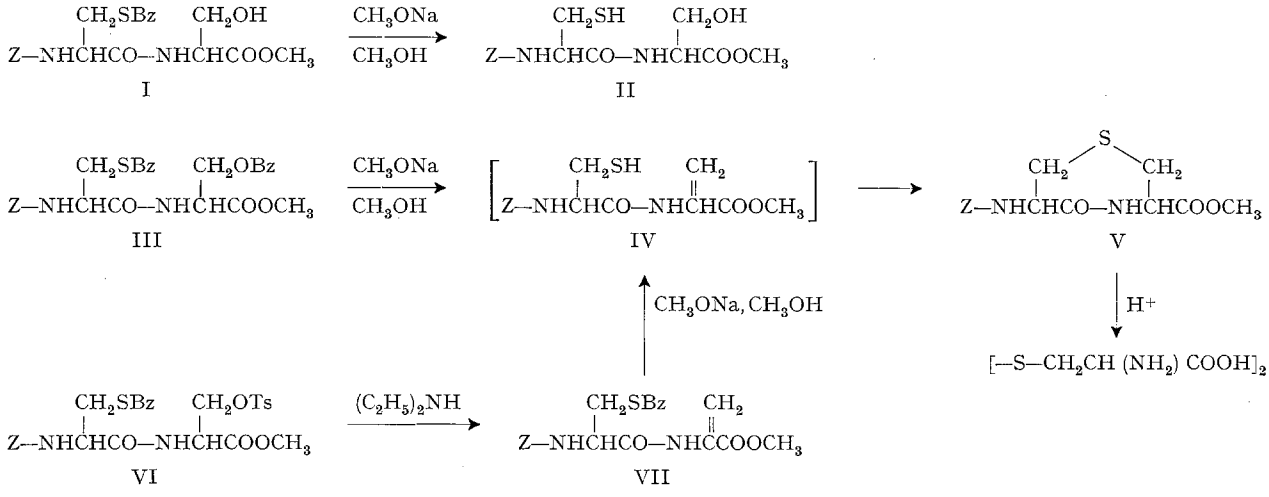
Umwandlung von Cysteinylserin in Lanthionin

Im Verlauf unserer Arbeiten über den Schutz von Aminosäure-Seitenketten bei Peptidsynthesen sind wir folgendem Fall begegnet.

Bei der Umesterung von N-Benzyloxycarbonyl–S–benzoyl–L–cysteinyl–L–serinmethylester (I, Smp. 174°, $[\alpha]_D^{31} = -36.4°$ in Dimethylformamid-DMF) mit methanolischer Natriummethylatlösung bildet sich erwartungsgemäss[1] der S-benzoyl-freie Ester II; wird dagegen der entsprechende S,*O*-Dibenzoylester III (Smp. 147°, $[\alpha]_D^{31} = -41.3°$ in DMF) auf dieselbe Weise behandelt, so wird der Serinanteil durch β-Abspaltung in Aminoacrylsäurederivat umgewandelt, während sich die gleichzeitig in Freiheit gesetzte SH-Gruppe an der entstandenen Doppelbindung addiert unter Bildung von N-Benzyloxycarbonylderivaten des L-Cyclolanthionylmethylesters (Va, Smp. 170°, $[\alpha]_D^{31} = +1.2°$ in DMF) und des meso-Cyclolanthionylmethylesters (Vb, Smp. 284–285°, $[\alpha]_D^{31} = -84.1°$ in Dimethylsulfoxyd). Die beiden Stereoisomere Va und Vb entstehen auch, sogar in besserer Ausbeute, wenn man den aus dem S-Benzoyl-*O*-tosyldipeptidester VI (Smp. 120°, $[\alpha]_D^{31} = -37.8°$ in DMF) in bekannter Weise[2] durch β-Abspaltung erhältlichen N-Benzyloxycarbonyl-S-benzoyl-L-cysteinyl-aminoacrylsäuremethylester VII mit methanolischer Natriummethylatlösung behandelt. Anscheinend ist IV als Zwischenprodukt bei der Umwandlung von III → V zu betrachten. Entcarbobenzoxylierung und anschliessende Hydrolyse von Va und Vb liefert L- bzw. meso-Lanthionin.

Der obige Reaktionsverlauf erinnert an die Bildung von Lanthionin bei der Hydrolyse von alkali-vorbehandelter Wolle[3] etc. und von Insulin[4], sowie an die Synthese von Lanthionin durch Wechselwirkung von Cystein und Acetylaminoacrylsäure in stark alkalischer Lösung[5]. Schliesslich möchten wir darauf hinweisen, dass Cyclolanthionylpeptide ein integrierender Bestandteil von Naturprodukten, z.B. Nisin, bilden[6].

Nachtrag bei der Korrektur: die Substanz von Smp. 284–285° hat sich inzwischen als ein dimeres von Vb mit einem 14gliedrigen Ringsystem erwiesen.



Z = $C_6H_5CH_2OCO$; Bz = C_6H_5CO–; Ts = p-$CH_3C_6H_4SO_2$

Summary. N-Benzyloxycarbonyl-S-benzoyl-L-cysteinyl-*O*-benzoyl-*L*-serine methyl ester is transformed to N-benzyloxycarbonyl derivatives of cyclo-L-lanthionyl methyl ester and of cyclo-meso-lanthionyl methyl ester.

L. ZERVAS und N. FERDERIGOS

Laboratorium für Organische Chemie, 13A Navarinou Street Universität Athen, Athen 144 (Griechenland), 14. August 1972

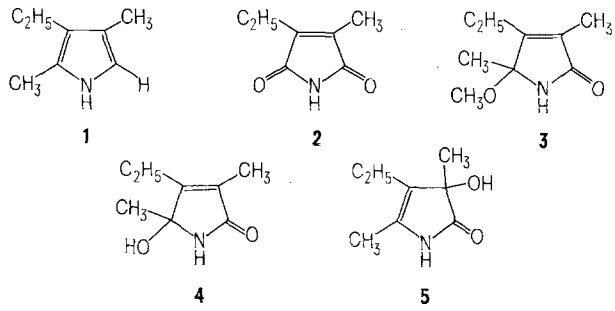
[1] L. ZERVAS, I. PHOTAKI und N. GHELIS, J. Am. chem. Soc. *85*, 1337 (1963).
[2] I. PHOTAKI, J. Am. chem. Soc. *85*, 1123 (1963).
[3] M. J. HORN, D. B. JONES und S. J. RINGEL, J. biol. Chem. *138*, 141 (1941).
[4] V. DU VIGNEAUD, G. B. BROWN und R. W. BOSNES, J. biol. Chem. *141*, 707 (1941).
[5] A. SCHÖBERL und A. WAGNER, Z. physiol. Chem. *304*, 97 (1956).
[6] E. GROSS und J. L. MORELL, J. Am. chem. Soc. *93*, 4634 (1971).

The Dye Sensitized Photooxygenation of Kryptopyrrole

In connection with chemical investigations into an increasingly widely applied phototherapy for neonatal jaundice[1,2] there has been a renewed interest in the behavior of monopyrroles during photosensitized oxygenation[3]. One of the most easily synthesized[4] and biologically important monopyrroles[5], kryptopyrrole (**1**), has been periodically examined following auto-oxidation[6,7], after which the principal isolated products were found to have dimeric structures[7]. However, there have been no reports on the photooxygenation of **1**. Because of their possible relationship to or existence as biological metabolites and their structural novelty, we wish to report on the photooxidation products of **1**.

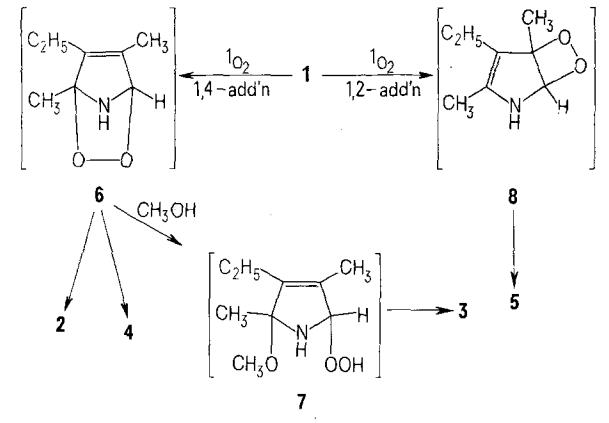
The photooxidation reaction was conducted in a water-cooled immersion apparatus containing a dilute (0.84 mmole %) methanolic solution of kryptopyrrole (**1**)

and 3.2 mg % of Rose Bengal ($^1O_2$ sensitizer). Irradiation[8] was continued for a period of 4 h while a slow stream of oxygen was bubbled through the solution. After evaporation of methanol from the reaction mixture, the residue was column chromatographed on silica gel (E. Merck, Darmstadt, 70–325 mesh ASTM) using gradient elution (chloroform-ether-ethyl acetate-acetone). A more complete separation of the eluted material was achieved using preparative thin layer chromatography (TLC) (Silica gel F, M. Woelm, Eschwege, 1 mm, diethyl ether) to give four main components, **2** (Rf 0.91) **3** (Rf 0.55), **4** (Rf 0.27) and **5** (Rf 0.41); theor. yields: 3, 14, 16 and 18% respectively.



1 **2** **3** **4** **5**

The structure of ethylmethylmaleimide (**2**) was confirmed by its TLC and spectroscopic [IR-, NMR- and mass spectrum[9]] identity to a known sample[10]. The expected methoxylactam (**3**) m.p. 84–5°, was identical to the 4-ethyl-5-methoxy-3, 5-dimethyl-3-pyrrolin-2-one isolated during the course of another work[11]. It exhibited a mass spectrum: *m/e* (relative intensity) 169.1105 [M^+, $C_9H_{15}NO_2$] (15%), 154 [$M-CH_3$] (11%), 140 [$M-C_2H_5$] (28%), 138 [$M-OCH_3$] (100%) and 122 (25%); NMR spectrum: δ (CCl_4) 1.14 (3H, t, CH_3), 1.48 (3H, s, CH_3), 1.79 (3H, s, CH_3), 2.24 (2H, q, CH_2), 2.95 (3H, s, OCH_3), and 7.68 (1H, br, NH) ppm.; and IR-spectrum: ν_{max} (KBr) 1689 (C=O) cm^{-1}. The hydroxylactam structure (**4**), m.p. 135–137.5°, was established by its mass spectrum: *m/e* (relative intensity) 155.0943 [M^+, $C_8H_{13}NO_2$] (27%), 140



[$M-CH_3$] (54%), 138 [M–OH] (19%), 126 (100%) and 122 (32%); NMR-spectrum: δ ($CDCl_3$), 1.17 (3H, t, CH_3), 1.51 (3H, s, CH_3), 1.73 (3H, s, CH_3), 2.35 (2H, q, CH_2), 2.90–3.50 (1H, br, OH) and 6.83 (1H, br, NH) ppm.; and IR-spectrum: ν_{max} (KBr) 1670 (C=O) cm^{-1}. The closely related structure of **5** was determined from its mass spectrum: *m/e* relative intensity) 155.0941 [M^+, $C_8H_{13}NO_2$] (7%), 140 [$M-CH_3$] (10%), 138 [M–OH] (100%), 126 (25%) and 122 (17%); NMR-spectrum: δ ($CDCl_3$) 1.16 (3H, t, CH_3), 1.51 (3H, s, CH_3), 1.80 (3H, s, CH_3), 2.34 (2H, q, CH_2), 7.17 (1H, br, NH) ppm.; and IR-spectrum: ν_{max} (KBr) 1701 (C=O) and 1625 (C=C) cm^{-1}. The close spectroscopic similarity of **4** and **5** rendered the structural assignments difficult; however, the lower carbonyl infrared stretching frequency of **4** agrees best with other 3-pyrrolin-2-ones and the higher value of **5** agrees with other unconjugated 4-pyrrolin-2-ones[12] and is akin to that observed in unsaturated γ-lactones[13]. Moreover, in **5** the C-3 methyl group is shielded by the adjacent carbonyl group and appears at slightly higher field (δ 1.80 ppm) than the corresponding C-5 methyl group of **4** (δ 1.73 ppm).

The formation of imides during pyrrole photooxidation has already been noted when both β-positions are alkylated[14,15], and they presumably originate from an *endo*-peroxide intermediate, e.g. **6** → **2** (Scheme). Methoxylactam **3** was also an expected product following methanolysis of *endo*-peroxide **6** with subsequent decomposition of hydroperoxide **7**. This is an expected reaction for alkylated pyrroles[3,14–16] and is well documented for furans[17]. Although the formation of hydroxylactams (e.g. **4**) has been observed whenever the pyrrole had one α-position alkylated and the remaining α-position unsubstituted[15,16], the OH group was always located at an α-position of the pyrrole ring. The product presumably arises via an *endo*-peroxide (e.g. **6**). Two possible mechanistic routes have been discussed briefly[16]. In marked departure from the behavior of related alkyl pyrroles, photooxidation of kryptopyrrole leads to a comparatively high yield of the unexpected isomeric hydroxylactam (**5**) in addition to the anticipated isomer (**4**). It is interesting to note that Höft, Katritzky and Nesbit[7] first reported a structure akin to **5**, *viz.* 3-hydroxy-3, 5-dimethyl-4-pyrrolin-2-one, as an autooxidation product of 2,4-

[1] D. A. Lightner and G. B. Quistad, Fedn. Europ. Bioch. Soc. (FEBS) Lett. *25*, 94 (1972). – J. D. Ostrow, Semin. in Hematol. *9*, 113 (1972).

[2] For a recent summary see D. Bergsma, C. Y.-Y. Hsia and C. Jackson, *Bilirubin Metabolism of the Newborn* (Williams and Wilkins Co., Baltimore 1970).

[3] For leading references see D. A. Lightner and L. K. Low, Chem. Commun. *1972*, 625.

[4] H. Fischer, Org. Synth., Colln. *3*, 513 (1955). An improved version of this synthesis involves concurrent Wolff-Kishner reduction, ester saponification and decarboxylation by heating ethyl 3-acetyl-3, 5-dimethyl-pyrrole-2-carboxylate with hydrazine and KOH in diethylene glycol.

[5] H. Fischer and H. Orth, *Die Chemie des Pyrrols* (Akademische Verlagsgesellschaft mbH, Leipzig 1934), Vol. 1, p. 47.

[6] W. Metzger and H. Fischer, Liebigs Annln. Chem. *1*, 527 (1937).

[7] E. Höft, A. R. Katritzky and M. R. Nesbit, Tetrahedron Lett. *1968*, 2028. *1967*, 3041,

[8] Westinghouse tungsten-halogen quartz lamp, 120 V, 500 W, No. 500 Q/LC run at 100 V.

[9] All mass spectra were determined on a CEC MS 21-491 or AEI MS-9 mass spectrometer; all NMR spectra were run on a Varian T-60 instrument; IR-spectra were recorded using a Perkin-Elmer 421 spectrometer.

[10] We wish to thank Dr. Z. Petryka, Northwestern Hospital, Minneapolis, Minn. for a generous sample of methylethylmaleimide.

[11] D. A. Lightner and G. B. Quistad, unpublished observations on the photooxidation of pyrrole aldehydes.

[12] L. K. Low, Masters Dissertation, UCLA (1972) and reference[3].

[13] R. E. Rosenkranz, K. Allner, R. Good, W. V. Philipsborn and C. H. Eugster, Helv. chim. Acta *46*, 1259 (1963). – K. Nakanishi, *Infrared Absorption Spectroscopy* (Holden-Day, San Francisco 1962), p. 44.

[14] G. B. Quistad and D. A. Lightner, Chem. Commun. *1971*, 1099. – D. A. Lightner and G. B. Quistad, Angew. Chem. *84*, 216 (1972).

[15] G. B. Quistad and D. A. Lightner, Tetrahedron Lett. *1971*, 4417.

[16] D. A. Lightner and L. K. Low, J. heterocycl. Chem. *9*, 167 (1972).

dimethylpyrrole and later revised the structure to the α-hydroxy isomer, 5-hydroxy-3, 5-dimethyl-3-pyrrolin-2-one. The only reported product of autooxidation of kryptopyrrole is a dimer[7]. We were thus quite surprised to discover **5** as a photoproduct of **1**, especially because none of the equivalent was product detected following photooxidation of the related 3, 4-diethyl-2-methylpyrrole[15]. At present the only other instance of a similar product originating from photooxygenation of a pyrrole may be found in the photooxidation of 3-methylpyrrole in methanol[3] from which low yields of both 3-hydroxy-3-methyl-4-pyrroline-2-one and 3-methoxy-3-methyl-4-pyrrolin-2-one were isolated among other products. For kryptopyrrole as well as 3-methylpyrrole, we propose a dioxetane intermediate (e.g. **8**) arizing from 1, 2-cycloaddition of $^1O_2$ to one of the enamine-like double bonds[18] of the pyrrole. We could find no analogous products arizing from attack of $^1O_2$ at the other double bond of **1**, nor could we detect a methoxylactam corresponding to **5**. We presume that an intramolecular rearrangement in **8** leads to **5**. Such a reaction course is abnormal in that similar dioxetane intermediates lead mainly to carbonyl products after C–C bond clevage[19], and apparent solvolysis products are usually minor components[19,20]. Further work on the mechanistic details of these reactions and studies on the photooxidation of other alkylated pyrroles are currently under investigation in our laboratories.

Zusammenfassung. Die durch Rose Bengal sensibilisierte Photooxygenierung des Kryptopyrrols in Methanol ergab Äthylmethylmaleimid, 4-Äthyl-5-methoxy-3, 5-dimethyl-3-pyrrolin-2-on, 4-Äthyl-5-hydroxy-3, 5-dimethyl-3-pyrrolin-2-on und 4-Äthyl-3-hydroxy-3, 5-dimethyl-4-pyrrolin-2-on.

D. A. LIGHTNER[21] and D. C. CRANDALL

Department of Chemistry,
Texas Tech University, P.O. Box 4260,
Lubbock Texas USA 79409, 5 December 1972.

[17] K. GOLLNICK and G. O. SCHENCK, *1,4-Cycloaddition Reactions*, (Ed. J. HAMER, Academic Press, New York 1967).
[18] C. S. FOOTE and J. W.-P. LIN, Tetrahedron Lett. *1968*, 3267.
[19] For leading references see D. R. KEARNS, Chem. Rev. *71*, 395 (1971).
[20] W. FENICAL, D. R. KEARNS and P. RADLICK, J. Am. chem. Soc. *91*, 3396 (1969).
[21] The authors wish to thank the National Science Foundation (No. GP-32483X and No. GP-35699X) and the National Institute of Child Health and Human Development. US Public Health Service (No. HD-07358) for generous support of this work. One of us (DCC) acknowledges receipt of a National Science Foundation Undergraduate Fellowhsip and a Pesident's Undergraduate Fellowship at the University of California, Los Angeles. We thank Miss ELISABETH IRWIN for determining all high resolution mass spectra reported in this work.

A Cholinesterase from Bean Roots and its Inhibition by Plant Growth Retardants

Acetylcholine (ACh) levels have been shown to be related to phytochrome-mediated processes in several plant systems[1,2], which suggested that a cholinesterase (ChE) might play a regulatory role in plant development. The occurrence of ACh-hydrolyzing activity of plant extracts has been reported in several works[3–5], but a detailed characterization of the enzyme(s) involved is still missing, and the question of whether or not ChE and especially acetylcholinesterase (AChE) exist in plants has not yet been answered. The present paper describes the purification and characterization of a ChE with high affinity for ACh from mung bean (*Phaseolus aureus*) roots and the effects of various plant growth retardants on the activity of the bean ChE.

Materials and methods. The ChE was purified from roots of 12-day-old light-grown seedlings. The roots were first extracted with 10 m*M* potassium phosphate buffer, pH 7.0, to remove soluble proteins and the ChE was then extracted from the plant residue with 4% $(NH_4)_2SO_4$ (w/v) in phosphate buffer. After concentration with $(NH_4)_2SO_4$ at 80% saturation followed by dialysis, the enzyme was further purified on a Sephadex G-200 column. Overall purification was 36-fold.

ChE activity was determined by the method of ELLMAN et al.[6] using thiocholine esters as a substrate, and

[1] M. J. JAFFE, Plant Physiol. *46*, 768 (1970).
[2] E. HARTMANN, Planta, Berl. *100*, 159 (1971).
[3] W. D. DETTBARN, Nature, Lond. *194*, 1175 (1962).
[4] A. TZAGOLOFF, Plant Physiol. *38*, 207 (1963).
[5] O. J. SCHWARTZ, M. Sc. thesis, North Carolina State University (1967).
[6] G. L. ELLMAN, K. D. COURTNEY, V. ANDRES and R. M. FEATHERSTONE, Biochem. Pharmac. *7*, 88 (1961).

Table I. Properties of bean root ChE

Characteristics	Bean ChE
Localization	Membrane-bound
Rate of hydrolysis of choline esters	Acetyl > propionyl = butyryl
Hydrolysis of non-choline esters	+
pH optimum with acetylthiocholine and ACh	8.5, 8.7
Shape of activity curve (ACh or acetylthiocholine as a substrate)	Bell-shaped (inhibition by excess substrate)
Km with ACh and acetylthiocholine (μM)	72, 84
Mol. Wt.	> 200,000 (evidence for smaller mol. wt. forms)
Concentration of eserine causing 50% inhibition (m*M*)	0.42
Concentration of neostigmine causing 50% inhibition (μ*M*)	0.60
Effect of choline	Stimulation