besitzt das Biliverdin hier denselben Bindungstyp wie die Gallenfarbstoffe in den Phykobilinen[4]; hierauf und nicht auf der chemischen Struktur der farbgebenden Gruppen dürfte die von LEMBERG[5] und FONTAINE[6] gefundene spektrale Ähnlichkeit mit dem Phykozyanin beruhen. Mit dem Lösen der N-Azyl-Bindung verschwindet die spektrale Besonderheit des Crenilabrus-Blaus: Das langwellige Maximum, das in 0,1 *n* H_2SO_4 bei 665 nm liegt, verschiebt sich beim Erhitzen der sauren Lösung (1 h auf 100 °C) auf 670–675 nm; dieselbe Verschiebung findet man beim Ansäuern des alkalibehandelten Chromoproteids. Freies Biliverdin absorbiert in 0,1 *n* H_2SO_4, wie in methanol. Salzsäure[7], maximal bei 670–680 nm.

Die beiden Bindungen sind sicher auch durch Säure (kalte konzentrierte Salzsäure bzw. heisse verdünnte Schwefelsäure) spaltbar, was für die Esterbindung durch den Chromsäureabbau des säurebehandelten Chromoproteids bewiesen wurde. Unter Säureeinwirkung reagieren aber die Vinylgruppen von Porphyrinen[8], Porphyrinogenen[9] und Gallenfarbstoffen[10] mit Thiolgruppen des Proteins. Diese Sekundärreaktion tritt auch beim Crenilabrus-Blau auf (vgl. Chromsäureabbau bei 100 °C), wobei gleichzeitig das Protein zu Peptiden hydrolysiert wird. Das Reaktionsprodukt ist weder wasserlöslich (wie das Ausgangsmaterial) noch chloroformlöslich (wie Biliverdin), löst sich aber in Amylalkohol und in methanolisierter Salzsäure. Aus ihm lässt sich durch nachträgliche Einwirkung von Alkali kein Biliverdin mehr gewinnen.

Summary. The blue biliprotein from the fins of the Mediterranean fish *Crenilabrus pavo* C.V. was analysed with respect to linkages between colouring matter (biliverdin IXα) and apoprotein. It was shown by chromic oxidation under various conditions and determination of degradation products that one of the outer and one of the inner rings of biliverdin are free. The remaining inner ring is bound to the apoprotein presumably by an ester bond, while the lability of the linkage between one outer ring and apoprotein corresponds to a N-acyl-bond.

W. RÜDIGER und L. ABOLINŠ

Institut für Biochemie der Universität des Saarlandes, Saarbrücken (Deutschland), und Zoophysiologisches Institut der Universität Uppsala (Schweden), 20. Januar 1969.

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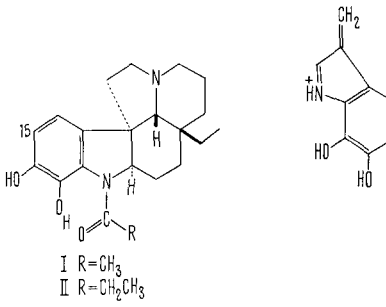
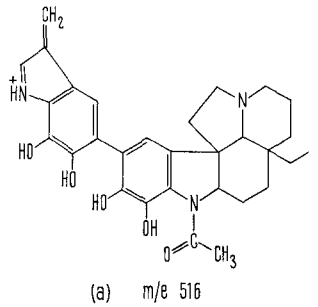
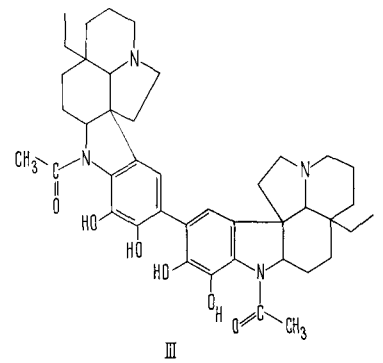
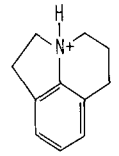
Alkaloids of *Aspidosperma melanocalyx* Muell-Arg.

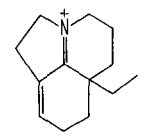
An investigation of the alkaloids of the bark of *Aspidosperma melanocalyx* Muell-Arg.[1] has resulted in the isolation of 2 principal bases. The first, extracted by chloroform from aqueous solution at pH 4, was identified as N-acetyl-16, 17-dihydroxyaspidospermidine (demethylaspidocarpine, 1)[2,3]. The presence of traces of the N-propionyl analogue of I (II) was indicated by mass spectrometry.











(b)

[1] Collected near Couto de Magalhães, Diamantina, Minas Gerais and Itaiaçu and Luisiana, Goiás, Brazil, by Mr. A. P. DUARTE who also identified the plant. Registered in the Rio de Janeiro Botanical Garden under Nos. 123334, 123335, and 125163.
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A second alkaloid extracted by chloroform at pH 5, crystallized directly and after recrystallization from hexane had m.p. 274–275°, $[\alpha]_D^{25}$ +108° (*c*, 1.01 in pyridine) I_2 then $Ce(SO_4)_2$ colour blue-green. High resolution mass spectrometry[4] established the molecular formula $C_{42}H_{54}N_4O_6$ (found, *M* 710.40631; calc., *M* 710.40431). IR-absorption at 2.92 and 6.11 μ indicated the presence of hydroxyl and strongly hydrogen-bonded amide groups as present in I. The UV-spectrum, λ_{max}^{EtOH} 236 and 297 nm (ε 19,500, 11,500), λ_{min}^{EtOH} 262 nm (ε 3550), $\lambda_{max}^{EtOH-NaOH}$ 243, 302 and 335 (infl.) nm (ε 16,900, 8600, 6800), was unaltered in acid. Its bathochromic shift in alkali shows the alkaloid to be phenolic. The NMR-spectrum (100 MHz in pyridine-D_5)[4] showed that the molecule was a true dimer having 2 CH_2CH_3 (triplet, 0.67 δ, J = 7 HZ) and 2 N–$COCH_3$ (singlet, 2.33 δ) groups and 2 protons in position 2 of an N-acylaspidospermidine skeleton (broad quartet, 4.20 δ). Only 2 aromatic protons were present and these appeared as a singlet at 7.11 δ. Acetylation with acetic anhydride-pyridine (24 h at 25 °C, 15 min at 90 °C) gave a tetra-*O*-acetate, m.p. 135–140°, whose mass spectrum showed M^+, 878 with successive losses of ketene to give peaks at 836, 794, 752 and 710. The lower region of the mass spectrum showed doubly charged ions of the same masses, but was otherwise similar to that of the original alkaloid. Taking these facts into account, the mass spectral fragmentation is in entire accord with structure III. M-28, M-42, M-43, m/e 124 (base peak), 138 and 152 indicated an N-acetyl aspidospermidine structure, but the indolic peak found at m/e 162 in the spectrum of I is shifted to m/e 516 in both III and its tetraacetate. The difference in mass corresponds to substitution by a unit of structure I (with loss of 2 hydrogen atoms) and this peak is assigned structure (a). 2 minor peaks at m/e 160 and 190 are interpreted as due to the aliphatic portion (b)[5].

The alkaloid (I) has aromatic proton absorptions at 6.76 and 7.17 δ (J = 8 Hz). In the dimer, the absorption at higher field is absent. This can be attributed to the C-15 proton and the link between the 2 halves is thus placed at C-15, 15′ in III. Comparison of the UV-spectrum of the dimeric alkaloid with that of I shows a substantial increase in intensity as well as a bathochromic shift, indicating considerable conjugation of the aromatic rings. Models show that the degree of coplanarity necessary for such conjugation could only be achieved if the linkage were 15-15′[6].

Zusammenfassung. In *Aspidosperma melanocalyx* wurde N-Acetyl-16, 17-dihydroxy-aspidospermin (Desmethyl-aspidocarpin) gefunden sowie dessen durch oxydative Dimerisierung entstandenes Produkt. Die Strukturaufklärung basiert auf physikalisch-chemischen Methoden.

E. C. Miranda and B. Gilbert[7]

Instituto Médico Legal do Estado da Guanabara, and
Centro de Pesquisas de Produtos Naturais,
Faculdade de Farmácia,
Rio de Janeiro ZC82 (Brazil), 14 January 1969.

[4] Mass spectra were measured on MS-9 and Atlas instruments by Dr. A. M. Duffield, and NMR-spectra by Dr. L. J. Durham of Stanford University.

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[6] Acknowledgment. Financial support has been provided by the Conselho Nacional de Pesquisas (Brazil) and the National Science Foundation, Grant No. GB 5389X.

[7] Author, to whom correspondence should be addressed.

Direct Synthesis of *p*-Methoxybenzyl Carbazate and *p*-Methoxybenzyloxycarbonylamino Acids Using *p*-Methoxybenzyl Chloroformate as Reagent[1]

Use of *p*-methoxybenzyloxycarbonyl [Z(OMe)] as a protective group in peptide synthesis was revived by the finding of Weygand and Hunger[2] in 1962 that the group can be removed easily with trifluoroacetic acid in the presence of anisole. The protective group can be introduced into amino acids with *p*-methoxybenzyl azidoformate[2], *p*-methoxybenzyl *p*-nitrophenyl carbonate[2], and *p*-methoxybenzyl 1-piperidyl carbonate[3]. However, a more convenient method should be developed for extensive use of this protective group.

This paper shows that *p*-methoxybenzyl chloroformate, which is easily prepared from anisyl alcohol and phosgene, can be used as a reagent to synthetize *p*-methoxybenzyl carbazate and Z(OMe)-amino acids directly by the Schotten-Baumann reaction. Practical conditions for synthesis of the chloroformate are also reported.

Materials and methods. A solution of anisyl alcohol (138 g, 1 mol) in dry ether (700 ml) was added dropwise with stirring to a solution of phosgene (200 g, 2 mol) in dry ether (1 l) over a period of 30 min at 0 °C. Then, the solution was concentrated to about 400 ml under reduced pressure in an ice-salt bath, and the resulting ether solution was used in following reactions as a solution of *p*-methoxybenzyl chloroformate without further purification.

This *p*-methoxybenzyl chloroformate solution (1 mol) was added drop-wise to a suspension of 80% hydrazine hydrate (625 g, 10 mol) in chloroform (1 l) at 0 °C over a period of 90 min with vigorous stirring. Stirring was continued for an additional 30 min at room temperature. The product was extracted with chloroform after addition of 2*N* NaOH (500 ml). The organic layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated to a residue, which was recrystallized from chloroform and *n*-hexane; the yield of *p*-methoxybenzyl carbazate was 167 g (85.2% from anisyl alcohol), m.p. 76 to 77 °C; reported m.p. 71–74 °C[2]. Anal. Found: C, 54.96; H, 6.19; N, 14.26%. Calcd. for $C_9H_{12}O_3N_2$: C, 55.09; H, 6.17; N, 14.28%.

[1] Presented at the 6th Symposium on Peptide Chemistry at Kyushu University, Fukuoka (Japan), 23 November 1968.

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