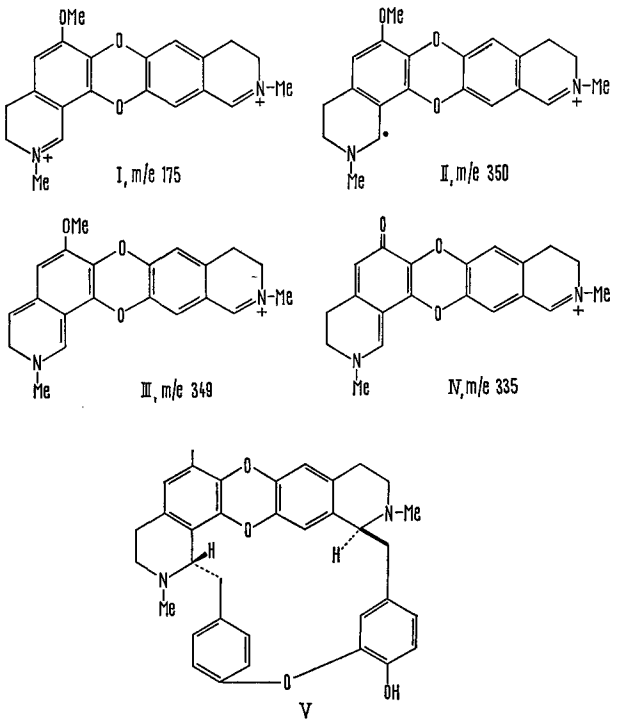

spectra of cocsulin and *O*-methylcocsulin as are also the abundant ions m/e 349 (III) and m/e 335 (IV) formed from II by the loss of a hydrogen atom and a methyl radical respectively. The presence of ions I, II, III and IV in the spectra of cocsulin and *O*-methylcocsulin is good evidence that the OMe group in cocsulin is attached to the tetrahydroisoquinoline portion and the hydroxyl to the benzylic half. Cocsulin can, therefore, be represented by the structure V with stereochemistry, since the stereochemistry of both centres in isotrilobine have been shown to have the S-configuration by examination of the fragments of Na/liq. NH_3 fisson[12].

Zusammenfassung. Ein neues *bis*benzylisochinolines Alkaloid vom Typ des Trilobins wird Cocsulin genannt, ($C_{35}H_{34}N_2O_5$), Schmp. 272–274°, $[\alpha]_D + 280°$, und ist aus Blättern und Stamm von *Cocculus pendulus* Diels isoliert und als Struktur V angegeben worden.

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Bromal, the Product of Reaction of Aspartic Acid with N-Bromosuccinimide

The oxidative decarboxylation of α-amino acids by N-bromosuccinimide (NBS) has been studied by several workers[1]. Reported products of this reaction are CO_2, N_2 and the aldehyde or nitrile corresponding to the decarboxylated amino acid[1]. NBS has also been used to specifically degrade N-acylated tyrosyl peptides by selective cleavage of the tyrosyl carboxyl-peptide bond[2] giving smaller peptides or free amino acids. When aspartic acid was treated with NBS, in the only investigation reported of this reaction, 2 moles of CO_2 (and no N_2) were evolved by KÖNIGSBERG et al.[1]; no attempt was made to characterize the decarboxylation product. Aspartic acid is oxidized at pH 9.4 by hypobromite to cyanoacetic acid and dibromoacetamide[3].

When solvent extracts of reaction mixtures of NBS and aspartic acid were bioautographed against *Staphylococcus aureus* or *Candida albicans*, we observed large bioactive zones. The antimicrobial agent was prepared by treating aspartic acid (2 g) in pH 3.0 citric acid-K_2HPO_4 buffer (88 ml) with NBS (2.72 g) at room temperature for 15 min. Extraction with organic solvents gave a substance which had a single NMR-peak at δ 8.52 in CCl_4. The IR-spectrum in this solvent had peaks at 3.54 and 5.71 μ, suggesting an aldehyde, and other peaks at 7.39, 9.94, and 10.17 μ. The material formed an unstable 2,4-dinitrophenylhydrazone that decomposed when refluxed with methanol, giving the 2,4-dinitrophenylhydrazone of methyl glyoxylate, mp 148.0–149.0[4]. This material was identified by its NMR-spectrum: ($CDCl_3$) δ 3.97 (s, 3), 7.12 (s, 1), 8.0–8.6 (m, 2), 9.17 (d, 1), 14.38 (broad, 1) and elemental analysis. The formation of this derivative and the spectra of the precursor suggested that the antimicrobial substance was bromal[5]. This was confirmed by comparison of the NMR- and IR-spectra with those of authentic bromal (Aldrich).

The approximate time-course of the reaction was studied under our reaction conditions by determining the diameter of the zone of inhibition around bioautographed discs dipped in BuOH extracts of replicate reaction mix-

Anti-bacterial activity of bromal

Organism tested	Squibb culture No.	M.I.C. μg/ml
Staphylococcus aureus 209p	1276	23.9
Escherichia coli	2975	47.8
Proteus vulgaris	8504	20.5
Pseudomonas aeruginosa	3840	9.0
Salmonella schottmuelleri	3850	47.8
Mycobacterium tuberculosis, BCG	5516	4.5
Candida albicans	5314	95.6
Trichophyton mentagrophytes	2637	383

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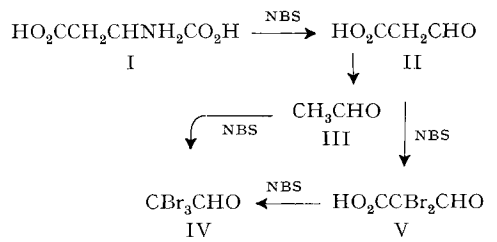
tures. Under these conditions the reaction had gone to completion in 3–5 min at room temperature, with about 75–85% of the final concentration of product present at 1 min. Variation of the pH of the citrate-phosphate buffer showed similar yields of BuOH-extractable antibacterial activity at pH 2.2 and 3.0, about 50% of this amount of activity at pH 4.0, a trace of activity at pH 5.0 and no discernible activity at pH 6.0, 7.0 and 7.9.

Again using the zone of inhibition as an assay, the yield of product from aspartic acid treated with various molar ratios of NBS was estimated. The quantity of NBS was varied from about 1 mole per mole of aspartic acid to about 25 moles per mole. The amount of product was about 34% at 1:1, about 62% at 2.5:1, and about 94% at 10:1, relative to 100% at 25:1.

Since no bioactive product was obtained when glutamic acid was substituted for aspartic acid as the substrate for NBS decarboxylation, this reaction might prove to be of value for the specific assay of aspartic acid without prior purification of an amino acid mixture or protein hydrolyzate.

The formation of bromal, IV, from aspartic acid, I, may follow the route shown below:



The first step, oxidative decarboxylation, is a characteristic reaction of amino acids with NBS[1]. The resulting formylacetic acid, II, would be expected to brominate rapidly at the methylene group to give V, followed by brominative decarboxylation to give bromal, IV. The alternative pathway of decarboxylation of II to acetaldehyde, III, followed by bromination of III to IV, is ruled out by the failure of acetaldehyde to form bromal on treatment with NBS under our conditions.

After the identity of our bioactive substance was ascertained, bromal was evaluated against a variety of microorganisms by a standard twofold tube dilution assay. The results are presented in the Table. Such data for this compound are not tabulated elsewhere, even though the germicidal properties of bromal have been recognized for over 50 years[6].

Zusammenfassung. Bromal wurde als ein Endprodukt der Reaktion zwischen Asparginsäure und N-Bromsuccinimid identifiziert und die antibakterielle Aktivität dieser Verbindung gegenüber einigen Mikroorganismen geprüft.

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The Tryptophans in Flavodoxin and Synthetic Flavinyl Peptides Characterized by Chemical and Photochemical Oxidations

Flavodoxin is a low molecular weight electron transfer flavoprotein first observed in iron-deficient *Clostridium pasteurianum*[1]. The isolated flavoprotein was observed to exhibit a shoulder in the flavin spectrum at 472 nm and was found to function as an electron carrier in the oxidation of pyruvate by the clastic system from the same microorganism with the subsequent formation of acetyl phosphate[2]. Analysis of clostridial flavodoxin revealed 4 tryptophan residues and 1 flavin mononucleotide (FMN) as tightly bound coenzyme with an apparent oxidation-reduction potential more negative than free flavin[3]. A similar FMN-dependent flavodoxin containing 4 tryptophans recently has been isolated from *Peptostreptococcus elsdenii*[4], and the oxidation-reduction properties of this system were examined[5].

The constant presence of tryptophan in flavodoxins suggests a possible role at the FMN-binding site, since of all the common amino acids, tryptophan complexes intermolecularly best with oxidized (quinoid) flavin in aqueous solution. Very tight intramolecular associations occur with synthetic flavinyl tryptophan peptides[6], wherein flavin fluorescence is markedly decreased[7]. Moreover, the generation of a red shoulder in the absorption spectrum of oxidized flavin resultant from association with tryptophan[6,7] is compatible with that seen in oxidized flavodoxins as is the lowering of observed oxidation-reduction potential which can result from selective decrease in the effective concentration of oxidized versus reduced flavin[8].

All of the above considerations led us to the present investigation of the nature of the tryptophan residues in flavodoxin by comparing their chemical and photochemical oxidizability with tryptophan in synthetic flavinyl tryptophan peptides and with tryptophan in the presence and absence of flavin.

Materials and method. Flavodoxin was purified from iron-deficient *C. pasteurianum* and the phosphoroclastic assay used[2]. Flavinyl tryptophans with chains of 1 and 5 methylene groups separating amide-linked tryptophan methyl ester from the N-10 position of the flavin nucleus were synthetized as described previously[6]. Chemical oxidations with N-bromosuccinimide (NBS) were carried out

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