(XIII) and a coupling to N-benzyloxycarbonyl-alanine (XIV) by a mixed anhydride step gave the tetrapeptide (XV). Hydrolysis by dilute alkali afforded N-benzyloxycarbonyl-alanyl-γ-*t*-butyl-glutaminyl-glycine (XVI). No evidence for glutaminyl hydrolysis was seen in this reaction.

The acid XVI was combined with the amine VI through a water-soluble carbodiimide to yield the S_{10-15} hexapeptide (XVII). Hydrogenation then furnished the amine (XVIII). A mixed anhydride coupling of N-benzyloxycarbonyl-ε-*t*-butyloxycarbonyl-lysine (XIX) with methyl O-*t*-butyl-serinate (XX) produced the dipeptide (XXI). Hydrolysis formed the corresponding acid (XXII), which was joined to the amine XVIII by a water-soluble carbodiimide in the presence of N-hydroxysuccinimide to give the octapeptide (XXIII). Hydrogenolysis then afforded the S_{7-15} fragment N-benzyloxycarbonyl-ε-*t*-butyloxycarbonyl-lysyl-O-*t*-butyl-seryl-alanyl-γ-*t*-butyl-glutamyl-glutaminyl-glycyl-glycyl-O-*t*-butyl-tyrosinamide (XXIV).

The preparation of the S_{3-7} portion was achieved in a different fashion. N-*t*-Butyloxycarbonyl-glycine (XXV) was joined to N-benzyloxycarbonyl hydrazine (XXVI) by the mixed anhydride reagent to give the protected hydrazide (XXVII). Cleavage with trifluoroacetic acid furnished the amine salt (XXVIII), which was combined with N-*t*-butyloxycarbonyl-glutamine (XXIX) by the mixed anhydride method to produce the dipeptide (XXX). Removal of the *t*-butyl group with trifluoroacetic acid formed the amine salt (XXXI). A mixed anhydride coupling with N-*t*-butyloxycarbonyl-γ-benzyl-glutamic acid (XXXII) gave the tripeptide (XXXIII). Treatment with trifluoroacetic acid afforded the amine salt (XXXIV), which on the addition of N-*t*-butyloxycarbonyl-asparagine *p*-nitrophenyl ester (XXXV) yielded the tetrapeptide (XXXVI). Removal of the *t*-butyl group in the usual manner furnished the amine salt (XXXVII), which on coupling to XXXV produced the pentapeptide (XXXVIII). Addition of trifluoroacetic acid then formed asparaginyl-asparaginyl-γ-benzyl-glutamyl-glutaminyl-glycyl-N^2-benzyloxycarbonyl-hydrazide trifluoroacetate (XXXIX).

The S_{1-2} unit was obtained in a simple fashion. A mixed anhydride coupling between N-benzyloxycarbonyl-O-*t*-butyl-serine (XL) and methyl β-*t*-butyl-aspartate (XLI) gave the dipeptide (XLII). Hydrogenation in the presence of one equivalent of pyridinium hydrochloride afforded the amine hydrochloride (XLIII) and the addition of acetic anhydride yielded the N-acetyl-dipeptide (XLIV). Hydrolysis with dilute base furnished N-acetyl-O-*t*-butyl-seryl-β-*t*-butyl-aspartic acid (XLV).

The acid XLV was combined by the mixed anhydride method with the amine salt XXXIX to produce the S_{1-7} heptapeptide (XLVI). Hydrogenation then formed N-acetyl-O-*t*-butyl-seryl-β-*t*-butyl-aspartyl-asparaginyl-asparaginyl-glutamyl-glutaminyl-glycyl hydrazide (XLVII). An organic azide coupling between XLVII and the amine XXIV gave the S_{1-15} pentadecapeptide (XLVIII). Treatment with trifluoroacetic acid afforded the deblocked trifluoroacetate salt (XLIX) and passage through an acetate ion-exchange resin yielded scotophobin acetate (L)[5].

Both scotophobin trifluoroacetate and scotophobin acetate were tested for the induction of a dark memory effect in untrained mice[6]. The former was lethal, but the latter possessed 2.5% of the activity of natural scotophobin at the 10.0 µg dosage level.

The lower biological behavior observed here, as well as the difference in R_f values (0.15 vs. 0.57), means that the postulated structure is in error, either in terms of sequence or in amino acid functionality. Work on several molecular variants is currently underway in order to clarify this situation[6].

Zusammenfassung. Es wurde ein biologisch interessantes Polypeptid mit der für Scotophobin vorgeschlagenen Sequenz synthetisiert. Da dieses nur eine geringe biologische Aktivität besass, wird angenommen, dass die für das natürliche Produkt vorgeschlagene Strukturformel nicht korrekt ist.

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Department of Chemistry, University of Washington, Seattle (Washington 98195, USA), 22 April 1971.

[5] All optically active amino acids are L; satisfactory microanalytical data were obtained for the new intermediates, while quantitative ammonia and amino acid assays were routinely performed on both blocked and deblocked products; thin-layer chromatography employed silica gel H as the support, chloroform-methanol (9:1) or acetic acid-*n*-butanol-water (1:4:1) for development, and iodine or ninhydrin for detection. Further experimental details will be given in the full paper.

[6] The pharmacology assays were furnished by Prof. G. Ungar, Baylor College of Medicine, Texas Medical Center, Houston (Texas, USA).

[7] We thank the National Institutes of Health (MH 19320) for the support of this work.

Periodic Acid as a New Oxidant for the Degradation of Bile Pigments. Isolation of a Biliverdine Type of Reaction Intermediate on Oxidation of Bilirubin with Periodic Acid[1]

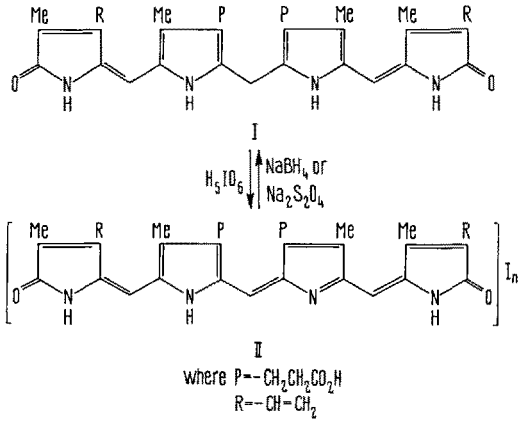
As part of a continued interest in new applications of periodic acid in organic chemistry[2], the study has now been extended to oxidation of bile pigments[3], particularly to samples of bilirubin that we are studying for the purpose of developing a standard reference material for clinical analysis. The bile pigments are usually analyzed by using degradation by chromic acid, either in 50% sulfuric acid (Willstätter[4] degradation) or at conrolled pH, the procedure of Rüdiger[5]. It has been found in this laboratory[6] that an aqueous solution of paraperiodic acid (H_5IO_6) may be used to degrade such porphyrins as hemin, hematoporphyrin, protoporphyrin, or chlorophyll, as well as such bile pigments as bilirubin (I), biliverdine (II, without iodine), urobilin, and other rubins, verdines, and violines. The co-solvents used in these degradations include dimethyl sulfoxide (Me_2SO, which shows superior solvating power for bile pigments and porphyrins), acetic acid, tetrahydrofuran, N,N-dimethylformamide, or acetone.

The final degradation products from bile pigments or porphyrins, usually mono-pyrrole derivatives, can be separated by extraction with ether or ethyl acetate into an acidic fraction (hematinic acid[7]) and a neutral fraction (2-ethyl-3-methyl-maleimide[7], methylvinylmaleimide[8] or, in certain instances, pyrroledialdehydes[5]). Identification

of the reaction products can be achieved either by mass spectrometry[8] or on thin-layer chromatograms (TLC), either by remission spectrometry[9] or by comparison of Rf values with those of authentic samples[5,10]. Chromatography of the degradation products by directly spotting samples of the reaction mixture gave good reproducibility; also, only as little as 5 to 8 μg of a bile pigment was needed for detection of imides by the chlorine-benzidine spray[11].

By the use of dimethyl sulfoxide as the co-solvent in the degradation of (I) with periodic acid, it was possible to isolate a reaction intermediate, a new biliverdine-iodine complex, to which a charge-transfer (structure II) has now been assigned.

A solution of bilirubin (0.2 g) in Me_2SO (10 ml) [λ_{max} 420 (sh) (ε_{mM} 48.9) and 458 nm (ε_{mM} 63.4)] prepared at 65 °C and cooled to 15 °C, was treated with aqueous periodic acid (4 *M*, 1.5 ml), causing an exothermic reaction; the deep-green solution was stirred for 8 min at 15–20 °C. Ice-cold water (20 ml) was then added, with stirring (10 min) to give a dark-green precipitate; this was collected, washed with water, and dried in a vacuum desiccator for 24 h; the yield of crude (II) was 0.14–0.18 g. A sample recrystallized from 1:2:1 (v/v) methanol-ethyl ether-pentane (room temperature) and then from 1:3 (v/v) methanol-ethyl ether (refrigerator), gave blue-green, microcrystalline (II) (45% yield from the crude product), m.p. 335–338° (dec., iodine vapor); TLC [microcrystalline cellulose with 4:3:3 (v/v) isopropyl ether-heptane-methyl alcohol] Rf 0.67 (green spot); usually, an impurity shows as a lilac spot at Rf 0.73. A sample[12] dried at 110 °C/0.1 torr for 2 h had C = 55.45; H = 4.59; I = 17.4; N = 7.45; calculated for $C_{33}H_{34}IN_4O_6$: C = 55.85; H = 4.83; I = 17.88; N = 7.89.



However, the proportion of iodine $(I)_n$ in (II) varied from one preparation to another, and this characteristic has been observed with certain iodine charge-transfer compounds[13,14]. Also, the X-ray diffraction pattern of (II) showed a low crystalline structure, and this also is a characteristic of certain iodine charge-transfer compounds[13,15]. The UV- and visible spectra of (II) [λ_{max}^{MeOH} 220, 272 (sh), 340, 372, and 660 nm] showed a maximum at 659–660 nm as compared to neutral biliverdine ($\lambda_{max}^{CH_3OH}$ 650 nm, ref.[16]) or protonated biliverdine ($\lambda_{max}^{0,1N\ HCl}$ 670 nm, ref. 16); other spectral data for (II): ν_{max}^{Nujol} 3190 (N-H), 1700 (C = 0), 1650, 1610 (–CH=CH–), 1580 (N-H, bending); 990, 945 and 908 cm^{-1} (–CH=CH_2); NMR (Me_2SO-d_6, TMS internal standard): δ (ppm) 9.70 (COOH), 8.30 (N-H), and 1.90 (CH_3).

Conductivity measurements[17] in CH_3OH suggested that the product is a weak electrolyte. The conductance of the solvent (CH_3OH) was 1.0 μohm^{-1} cm^{-1} and that of stock solution of 4.328 mg of (II) in 14.382 *g* of CH_3OH was 10.53 μohm^{-1} cm^{-1}. Potentiometric titration of a sample (in 50% aqueous (CH_3OH) produced a curve characteristic of a dibasic acid, with pK_1 4.1 ± 0.3, pK_2 7.3 ± 0.5, and an equivalent weight of 292 [after correction for non-tritable iodine present in the sample of (II)]. The mass spectrum of (II) at 150–170 °C did not show a parent-ion peak; however, it showed an ion peak at *m/e* 582 (5% of the base peak) and ion peaks at *m/e* 254 (100%) (I_2^+) and *m/e* 127 (90%) (I^+, HI), in addition to the expected[8] smaller fragments. The observed resistance of the peak at *m/e* 254 (I_2^+) to heat (250–300 °C) is a characteristic found with other iodine charge-transfer complexes.

Final verification of the structure[18] of (II) was obtained by its reconversion into bilirubin by reduction. Treatment of a methanol solution of part of the analytical sample of (II) with sodium hydrosulfite[19] ($Na_2S_2O_4$) in 0.2 *N* NaOH or with a calculated amount of sodium borohydride[20] in

[1] Part VIII of the Series: Periodic Acid, a Novel Oxidant. For some previous parts, see ref.[2].

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[12] An analytical sample of (II) was purified on a column of microcrystalline cellulose by successive elution with 1:3 (v/v) methanol-ethyl ether and 3:1 (v/v) methanol-ethyl ether. The green iodinated complex (II) was somewhat resistant to further oxidation at room temperature; however, on prolonged treatment with oxidant (4–7 days), the unsaturated bridges in (II) were ruptured to yield a yellowish solution. At 65 °C oxidation of (I) (0.1 g) in methyl sulfoxide (5 ml) with aqueous periodic acid (4 *M*, 0.8 ml) produced a yellow-orange solution in 30 min. In aqueous acetic acid the degradation of (I) usually requires 12 to 24 h (room temperature); however, in acetone it takes 2 to 3 h, but the mixture of products is more complex. Aqueous acetic acid is a preferred media for the degradation of (I) for a systematic study of the degradation products.

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[16] C. H. GRAY, A. LICHTANOWICZ-KULCZYCKA, D. C. NICHOLSON, and Z. PETRYKA, J. chem. Soc. 1961, 2264.

[17] The authors thank Dr. T. B. HOOVER for the conductivity measurements, potentiometric titrations and stimulating discussion.

[18] A likely structure of (II) is a donor-acceptor type involving in the C ring a nitrogen atom having a lone pair of electrons and one half of a molecular of iodine. A possible polymeric structure involving chains of cations of (II) with iodine anions is improbable because of the solubility of the compound and its chemistry (reduction).

[19] H. FISCHER and H. PLIENINGER, Hoppe Seyler's Z. physiol Chem. *274*, 231 (1942).

[20] Controlled reduction of the complex (II) with sodium borohydride gives some bilirubin (TLC); however, the reduction easily proceeds further and the use of a small excess of the reagent (which is difficult to quantitize) produces a dihydrobilirubin ($\lambda_{max}^{CHCL_3}$ 442–444 nm) and (by TLC) practically no mesobilirubin ($\lambda_{max}^{CHCL_3}$ 432–434 nm)[22]. II is easily converted into bilirubin on treatment with $Na_2S_2O_4$. Treatment of the complex with aqueous sodium hydrogen sulfite ($NaHSO_3$) also causes the removal of iodine.

methanol at room temperature, followed by careful acidification with 2 *M* hydrochloric acid (to pH 4–5 for $NaBH_4$ reduction), extraction with chloroform, and thorough washing of the extract with water (which is imperative) gives (I), $\lambda_{max}^{CHCl_3}$ 452 nm (ε_{mM} 60.4), in 60% yield. The bilirubin obtained was free from an impurity[21] that several commercial preparations of bilirubin were found to exhibit under 366 nm irradiation after TLC of ~50 µg of (I) on activated silica gel with 8:1:1 (v/v) benzene-N, N-dimethylformamide-glacial acetic acid. This impurity is seen as a pink-red fluorescence (366 nm irradiation) at Rf 0.81. Under visible light a yellow spot (bilirubin) at Rf 0.86 and a green zone (biliverdine ?) at Rf 0.64 are seen. The major proportion of the bilirubin remains at the origin on the silica gel plate. On polyamide, TLC with 1:3 (v/v) 3.3% aqueous ammonia-methanol[23] revealed a similar pink fluorescence (366 nm irradiation) at Rf 0.75, just ahead of the leading edge of the streaked bilirubin.

The interconversion of (I) into (II) opens the prospect of achieving controlled, stepwide structural changes of related bile pigments that display similar susceptibility to over-oxidation. The behavior of periodic acid toward (I) indicates structural lability of the I-O bond in the reagent, and this is observed with other compounds[2]. In addition, by use of periodic acid, it was found feasible to degrade porphyrins (for example, hematoporphyrin), whereas we observed a resistance of such compounds to degradation with chromic acid. Furthermore, the conversion of (I) into (II) and thence back into (I) provides a method for obtaining purified (I).

Zusammenfassung. Die Oxydation des Gallenfarbstoffs Bilirubin mit Perjodsäure in wässeriger Dimethylsulfoxid-Lösung führte zu einem neuen Biliverdin-Jod-Komplex, dem die Struktur eines «charge-transfer»-Komplexes erteilt wurde.

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National Bureau of Standards,
Washington (D.C. 20234, USA) 13 April 1971.

[21] It is interesting that the oxidation mixture from (I) contained a substantial proportion of an unknown component showing a strong blue fluorescence; a component having this property was found present also in controlled chromic acid oxidations, but Rüdiger et al.[5] did not report such an observation in their study of the degradation products of bilirubin and biliverdine derivatives. A component like this was also observed among the products of degradation of hemin with periodic acid. The unknown fluorescent component has the following Rf values on Silica Gel G: with 1:5:3 (v/v) cyclohexane-carbon tetrachloride-ethyl acetate, 0.76 ± 0.03, and with 4:1 (v/v) benzene-ethyl acetate, 0.70 ± 0.02. The unknown compound can be separated by use of a Florisil column (protected from light) with the latter solvent.

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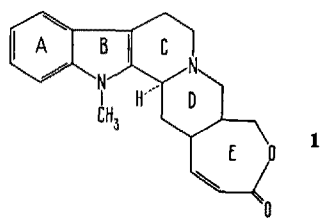
[24] The authors are most pleased to acknowledge the partial support of this work by the National Institute for General Medical Sciences (NIH).

Alcaloïde des Canneberges du Nouveau-Brunswick

L'extraction des canneberges (Vaccinium Oxycoccus) du Nouveau-Brunswick donne une fraction basique qui se compose de 19 bases différentes. La structure d'une de ces bases a été déterminée par l'étude des spectres: spectroscopie de masse (haute résolution), RMN, IR et UV. Nous avons trouvé que cette base possède un squelette semblable à certains alcaloïdes indoliques tel que la réserpine, la sempervinine, la yohimbine ou l'harméine[1–3]. Cette base, que nous avons appelé Cannagunine (**1**), possède la structure suivante:



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La diffraction de rayons X supporte notre proposition en plaçant l'hydrogène sur la jonction des noyaux C et D en position α.

Méthode. 20 kg de canneberges fraîches (novembre 1968) ont été sechés à la température ambiante pendant 7 mois (perte de ~45% d'eau); ensuite sechés pendant 48 h à une température de 60 °C et pulvérisés. La poudre a été sechée à une température de 60 °C (l'air). Nous avons obtenu 450 g du matériel sec. Ce matériel a été extrait à l'éther dans un appareil Soxhlet (séparation des graisses); ensuite extrait avec 3000 ml de méthanol. Une suspension brune a été filtrée et la solution a été concentrée. 500 ml d'eau ont ensuite été ajoutés et la solution a été acidifiée avec HCl à 10% (pH 2). La solution aqueuse a été d'abord entrainée à la vapeur d'eau (séparation du méthanol) et extraite avec l'ether et le chloroforme (séparation du matériel neutre et acide); puis alcalisée avec NaoH à 10% (pH 9) et de nouveau extraite avec l'éther (1000 ml) et le chloroforme (500 ml). Les extraits combinés ont été sechés avec $MgSO_4$ anhydre et évaporés. On a obtenu 2.3 g de matériel basique brut.

La chromatographie sur couches minces (CCM) (MN Silica Gel G) dans les quatre systèmes de solvants (benzène-acétone 4:1, benzène-éthanol 9:1, benzène-méthanol-ether 9:1:1, benzène-acétone-méthanol 9:1:1) a revélé la présence d'un minimum de 19 produits basiques différents. Une des bases – Rf 0.46 (benzène-éthanol 9:1) étant fortement absorbée dans UV – a été isolée par la CCMP (MN Silica Gel G, épaisseur: 1 mm) puis de nouveau purifiée par CCMP. On a obtenu 1.7 mg d'une base **1** dont 1.2 mg ont été utilisés pour l'identification.

Identification de la structure de la Cannagunine (**1**). Le spectre de masse haute résolution de la Cannagunine (**1**) a donné le poids moléculaire 322.1714 ± 0.0040 (corrigé[4] 322.1601) correspondant à la formule brutte $C_{20}H_{22}O_2N_2$. Cette formule représente un système de 10 cités d'insaturation. Le spectre UV (Beckmann, éthanol 95%) a dé-

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[3] H. Budzikiewicz, D. H. Williams et C. Djerassi, *Structure Elucidation of Natural Products by Mass Spectrometry* (Holden-Day, Inc., San Francisco 1964), Vol. 1, p. 77.

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