Communications

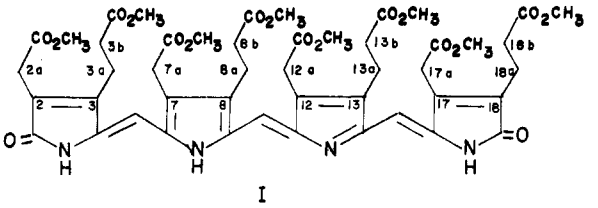
Total Synthesis of the Urobiliverdin Isomers. Identification of Bactobilin as Urobiliverdin I

Summary: The total synthesis of the five urobiliverdin isomers which can be derived from the oxidation of uroheme I and uroheme III was performed, and the natural bactobilin was found to be identical with urobiliverdin I. The identity was established by comparison of the high-resolution ^1H NMR spectra and by HPLC analysis.

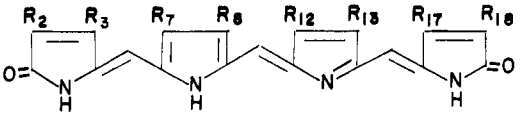
Sir: A new bile pigment possessing four acetic and four propionic acid side chains has been recently isolated from extracts of the anaerobic microorganisms *Clostridium tetanomorphum* and *Propionibacterium shermanii*.[1] The pigment, for which the name bactobilin was proposed,[1] was assigned the structure of a bilitriene of the urobiliverdin type, i.e., a bile pigment whose side chains correspond to those of the uroporphyrins (Chart I). Bactobilin is the first bile pigment detected in prokaryotes and is the only known biliverdin which cannot be derived from the enzymatic oxidation of iron protoporphyrin IX (protoheme).[2] Oxidative breakdown of the two uroporphyrin isomers known to be present in porphyrin metabolism[3] (uroporphyrin I and uroporphyrin III) could give rise to five urobiliverdin isomers[4] (Chart I). Analysis of the ^1H NMR spectra of bactobilin ruled out the structures IIIγ and IIIδ for this pigment, since bactobilin appeared to have one exo (α to the amide carbonyl group) and three endo (at the other positions of the pyrrole rings) propionic acid residues.[1]

In order to establish unambiguously the structure of bactobilin we have carried out the total synthesis of the five urobiliverdin isomers. This was achieved by the condensation of [5′-[(*tert*-butyloxy)carbonyl]-5-formyldipyrryl]methanes with α-unsubstituted [2-(*tert*-tubyloxy)carbonyl]dipyrryl]methanes in the presence of HBr to give the corresponding *b*-bilene hydrobromides (Scheme I), following procedures outlined to our previous work.[5] The *b*-bilene hydrobromides were oxidized to the corresponding urobiliverdin octamethyl esters with bromine and trifluoroacetic acid.[6] The octamethyl esters were purified by column chromatography on TLC silica using 4:4:2 chloroform/acetone/methanol as eluant and were finally repeatedly crystallized from chloroform/hexane. The yields of the oxidation reactions were about 10%.

Chart I. Urobiliverdin Isomers



I



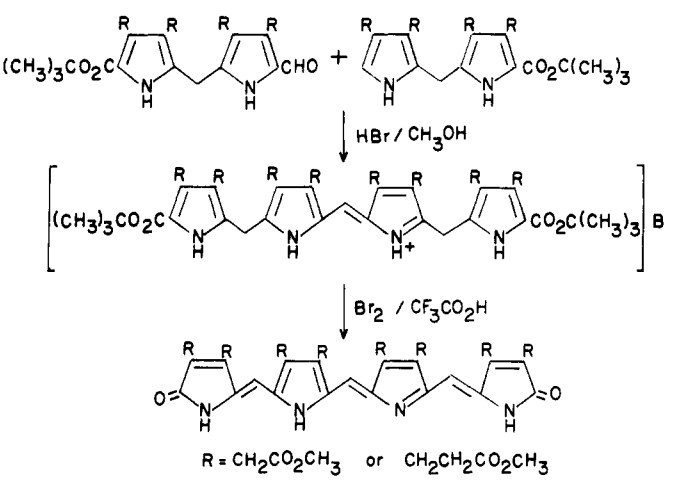
IIIα $R_2 = R_7 = R_{13} = R_{17} = CH_2CO_2CH_3$; $R_3 = R_8 = R_{12} = R_{18} = CH_2CH_2CO_2CH_3$

IIIβ $R_2 = R_8 = R_{12} = R_{17} = CH_2CO_2CH_3$; $R_3 = R_7 = R_{13} = R_{18} = CH_2CH_2CO_2CH_3$

IIIγ $R_3 = R_7 = R_{12} = R_{17} = CH_2CO_2CH_3$; $R_2 = R_8 = R_{13} = R_{18} = CH_2CH_2CO_2CH_3$

IIIδ $R_2 = R_7 = R_{12} = R_{18} = CH_2CO_2CH_3$; $R_3 = R_8 = R_{13} = R_{17} = CH_2CH_2CO_2CH_3$

Scheme I



R = $CH_2CO_2CH_3$ or $CH_2CH_2CO_2CH_3$

Table I. Electronic Absorption Spectra of the Urobiliverdin Isomers Octamethyl Esters[a]

isomer	λ_{max} (nm)	ϵ_{mM}	λ_{max} (nm)	ϵ_{mM}
I	372	51.3	641	9.6
IIIα	371	59.4	625	13.3
IIIβ	372	55.3	655	8.1
IIIγ	372	51.0	638	8.2
IIIδ	371	33.0	635	6.6

[a] Spectra were determined in $CHCl_3$.

The visible spectra of the isomeric octamethyl esters showed relatively important differences (Table I), which must be attributed to the superposition of spectra of various conformers in thermodynamic equilibrium. Although the spectra of the octamethyl esters of urobiliverdin

(1) Brumm, P. J.; Fried, J.; Friedmann, H. C. *Proc. Natl. Acad. Sci. (U.S.A.)* **1983**, *80*, 3943-3947.
(2) For reviews on bile pigments in nature, see: (a) Bennett, A.; Siegelman, H. W. In "The Porphyrins"; Dolphin, D., Ed; Academic Press: New York, 1979; Vol. 6A, pp 493-520. (b) Lightner, D. A.; ref 2a, pp 521-584. (c) McDonagh, A. F.; ref 2a, pp 257-292. (d) Scheer, H. *Angew. Chem. Int. Ed. Engl.* **1981**, *20*, 241-261. (e) Brown, S. B.; Holroyd, J. A.; Troxler, R. F.; Offner, G. D. *Biochem. J.* **1981**, *194*, 137-147.
(3) Frydman, R. B., Frydman, B.; Valasinas, A. In "The Porphyrins"; Dolphin, D., Ed.; Academic Press: New York, 1979; Vol. 6A, pp 1-123.
(4) For nomenclature of porphyrins and bile pigments, see: Bonnett, R. In "The Porphyrins"; Dolphin, D., Ed; Academic Press: New York, 1979; Vol. 1, pp 1-27.
(5) Díaz, L.; Valasinas, A.; Frydman, B. *J. Org. Chem.* **1981**, *46*, 864-867. Díaz, L.; Frydman, R. B.; Valasinas, A.; Frydman, B. *J. Am. Chem. Soc.* **1979**, *101*, 2710-2716. Díaz, L.; Buldain, G.; Frydman, B. *J. Org. Chem.* **1979**, *44*, 973-977. Valasinas, A.; Frydman, B. *J. Org. Chem.* **1976**, *41*, 2991-2994. Valasinas, A.; Levy, E. S.; Frydman, B. *J. Org. Chem.* **1974**, *39*, 2872-2877.
(6) Kishore, D.; Smith, K. M. *Tetrahedron* **1983**, *39*, 1841-1847.

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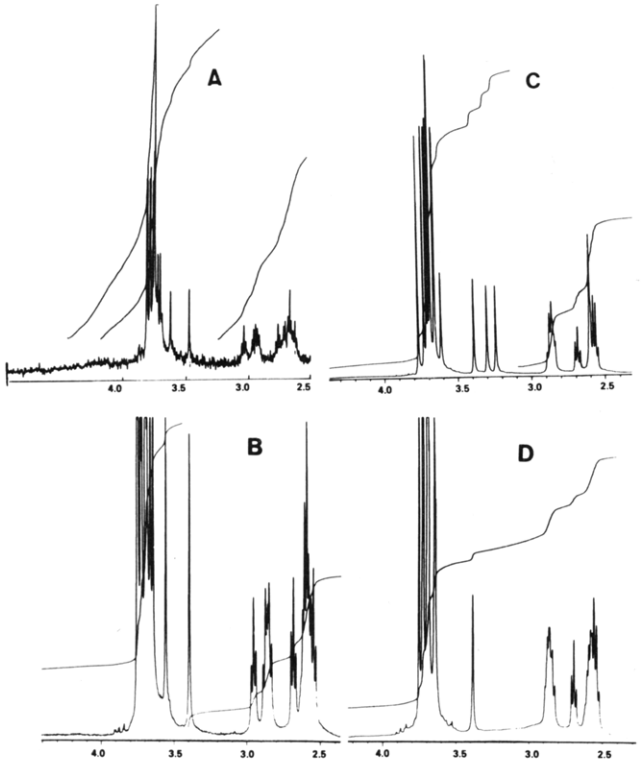

Figure 1. The 500-MHz ^{1}H NMR spectrum of bactobilin octamethyl ester (A). The 470-MHz ^{1}H NMR spectra of the octamethyl esters of urobiliverdin I (B); urobiliverdin IIIα (C); and urobiliverdin IIIβ (D). The 500-MHz spectrum was recorded on a University of Chicago built DS-1000 spectrometer equipped with a Nicolet 1180 data acquisition system. The 470-MHz spectra were recorded at 5000 Hz on a Nicolet 470-MHz (^{1}H) spectrometer with a 32 K memory and processed by a Nicolet computer. The spectra were obtained in $CDCl_3$. The chemical shifts are quoted in ppm downfield from Me_4Si, and only the 2.5–4.0-ppm region is shown.

I and bactobilin ($vis_{max}(CHCl_3)$, 641 nm) are very similar, differences in the low energy absorptions among the isomers are insufficient for reliable comparison purposes. However, comparison of the high-resolution ^{1}H NMR spectra of the isomeric urobiliverdin esters with that of bactobilin ester leaves no doubt that the natural bactobilin is urobiliverdin I (Figure 1). The differences in the chemical shifts of the methylene groups of the four acetate residues of the esters of urobiliverdin I, urobiliverdin IIIα, and urobiliverdin IIIβ in the 3.2–3.7-ppm region give a reliable basis for distinguishing between the isomers. A signal at 3.393 ppm in urobiliverdin I corresponds to the protons of the exo methylene 2a on the basis of the considerations made for the methyl residues of the biliverdin IX isomers.[7] A signal at 3.555 ppm was assigned to the methylene 17a (see Chart I), while the signals at 3.643 and 3.662 ppm were assigned to the other two acetic methylenes (7a and 12a). These chemical shifts are essentially the same in the bactobilin spectrum[8] and very different from those of the IIIα and IIIβ isomers (Figure 1). The spectra of urobiliverdins IIIγ and IIIδ (not shown in Figure 1) fully support the considerations which excluded these isomers as possible structures for bactobilin.[1] In the IIIγ isomere the exo propionic methylene protons at the 2a and 18a positions moved upfield and are coincident with the signals of the propionic methylens at 2b, 8b, 13b, and 18b; while only two endo methylenes remained at low field (2.891 and 2.793 ppm), corresponding to the 8a and 13a positions. In the IIIδ isomer no high field (above 2.840 ppm) exo propionic methylene protons corresponding to the 2a and 18a positions were found (see paragraph at the end of the paper about supplementary material).

Urobiliverdins I, IIIa, and IIIβ could be separated by HPLC[9] and the identity of urobiliverdin I and bactobilin was further confirmed by this method.

The presence of urobiliverdin I in bacteria was unexpected since all the natural biliverdins in eukaryotes are type III isomers.[2] Heme oxygenase is certainly not involved in the oxidation of hypothetical uroheme I since extensive studies on the specificity of this enzyme have shown that neither hemins of type I nor octacarboxylic hemins are substrates of the enzyme.[10] Uroheme III was recently chemically oxidized in very low yields to a mixture of the urobiliverdin isomers,[11] but the enzymatic results suggest that an uroporphyrinogen (a tetrahydroporphyrin) rather than a uroporphyrin is the precursor of bactobilin.[1] The fact that the synthetic urobiliverdins are excellent substrates of biliverdin reductase[12] adds relevance to the physiological significance of bactobilin.

Acknowledgment. We are grateful to Professor Josef Fried (Department of Chemistry, The University of Chicago) for the spectrum of natural bactobilin, to Professor Ching-jer Chang (Department of Medicinal Chemistry, School of Pharmacy, Purdue University) for running the 470-MHz spectra, and to Mr Sergio Rosé (Facultad de Farmacia y Bioquimica, Universidad de Buenos Aires) for the HPLC analysis of the urobiliverdin isomers. This work was made possible by Grant GM-11973 of the National Institutes of Health.

Registry No. I (*b*-bilene hydrobromide derivative), 96246-54-7; IIIα (*b*-bilene hydrobromide derivative), 96246-55-8; IIIβ (*b*-bilene hydrobromide derivative), 96246-56-9; IIIγ (*b*-bilene hydrobromide derivative), 75993-24-7; IIIδ (*b*-bilene hydrobromide derivative), 96246-57-0; urobiliverdin I, 96246-58-1; urobiliverdin I octamethyl ester, 96246-52-5; urobiliverdin IIIα octamethyl ester, 88815-22-9; urobiliverdin IIIβ octamethyl ester, 88815-23-0; urobiliverdin IIIγ octamethyl ester, 88815-24-1; urobiliverdin IIIδ octamethyl ester, 88815-25-2; [5′-[(*tert*-butyloxy)carbonyl]-4′,3-bis(methoxycarbonylmethyl)-3′,4-bis(methoxycarbonylethyl)-5-formyldipyrryl]methane, 84315-35-5; [5′-[(*tert*-butyloxy)carbonyl]-4′,4-bis(methoxycarbonymethyl)-3′,3-bis(methoxycarbonylethyl)-5-formyldipyrryl]methane, 84315-36-6; [5′-[(*tert*-butyloxy)-carbonyl]-3′,3-bis(methoxycarbonylmethyl)-4′,4-bis(methoxycarbonylethyl)-5-formyldipyrryl]methane, 75975-56-3; [2-[(*tert*-butyloxy)carbony]-4′,3-bis(methoxycarbonylmethyl)-3′,4-bis-(methoxycarbonylethyl)dipyrryl]methane, 61637-70-5; [2-[(*tert*-butyloxy)carbonyl]-3′,3-bis(methoxycarbonylmethyl)-4′,4-bis-(methoxycarbonylethyl)dipyrryl]methane, 75975-55-2; [2-[(*tert*-butyloxy)carbonyl]-4′,4-bis(methoxycarbonylmethyl)-3′,3-bis-(methoxycarbonylethyl)dipyrryl]methane, 96246-53-6.

(7) Bonnett, R.; McDonagh, A. F. *J. Chem. Soc., Perkin Trans. 1* **1973**, 881–888.

(8) The chemical shifts of the methylene protons of the acetate residues were not mentioned in the original report[1] of the ^{1}H NMR spectrum of bactobilin.

(9) The separation was carried out on a Perkin-Elmer LC-75 (series 10) instrument and was achieved by using a Lichrosorb RP-18 column (250 × 4 mm) and 2% acetonitrile in 0.05 M sodium citrate buffer solution (pH 7) as eluant. Flow rate was 0.7 mL/min at 1200 psi. The urobiliverdin peaks were detected at 372 nm.

(10) Frydman, R. B.; Awruch, J.; Tomaro, M. L.; Frydman, B. *Biochim. Biophys. Res. Commun.* **1979**, *87*, 928–935. Frydman, R. B.; Tomaro, M. L.; Buldain, G.; Awruch, J.; Díaz, L.; Frydman, B. *Biochemistry* **1981**, *20*, 5177–5182. Bezzano, I.; Tomaro, M. L.; Buldain, G.; Frydman, R. B. *Biochim. Biophys. Acta* **1982**, *704*, 261–266. Tomaro, M. L.; Frydman, R. B.; Frydman, B.; Pandey, R. K.; Smith, K. N. *Biochim. Biophys. Acta* **1984**, *791*, 342–349.

(11) Bendikt, E.; Kost, H. P. *Z. Naturforsch. C: Biosci.* **1983**, *38c*, 753–757.

(12) Tomaro, M. L.; Frydman, R. B.; Awruch, J.; Valasinas, A.; Frydman, B.; Pandey, R. K.; Smith, K. M. *Biochim. Biophys. Acta* **1984**, *791*, 350–356.

Supplementary Material Available: Full ^1H NMR data (Table and spectra) of the urobiliverdin isomers, as well as their melting points, mass spectral data of urobiliverdin I octamethyl ester, and r_T of the HPLC separation (7 pages). Ordering information is given on any current masthead page.

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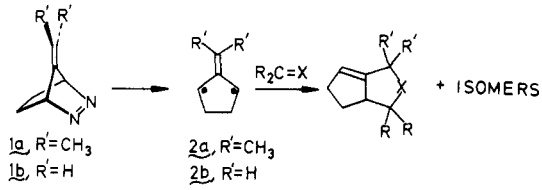
Use of Heteroatom-Containing π Systems as Diylophiles in the Intermolecular 1,3-Diyl Trapping Reaction. Construction of Heterocyles

Summary: Reactions illustrating the intermolecular cycloaddition of heteroatom-containing π systems and alkynes to 2-alkylidenecyclopentane-1,3-diyls are described.

Sir: Unlike [4 + 2] cycloaddition reactions wherein a wide range of dienophiles have been used,[1] the diyl trapping reaction has been restricted to examples wherein the trapping agent (the diylophile) consists of a simple C–C π bond, most often substituted with an electron-withdrawing group.[2] We now report that *diylophiles which incorporate heteroatoms* (viz., C=O, C=N, and C=S) *can also serve as trapping agents*, thereby extending the scope of the diyl trapping reaction and also suggesting its use in the construction of heterocycles.[3]

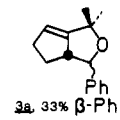
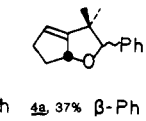
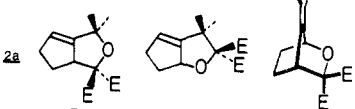
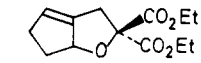
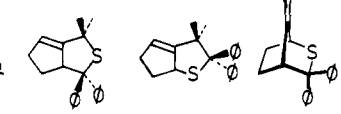
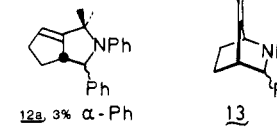
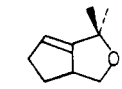
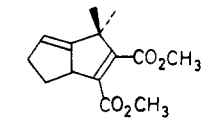
Most of our attention has focused upon the use of the dimethyl diazene **1a** (R′ = CH_3). It, when heated in refluxing THF, presumably serves as a precursor to diyl **2a**, which in turn is trapped with a variety of diylophiles $R_2C{=}X$ (X = O, N, S); thereby leading to the formation



1a, R′=CH_3 1b, R′=H 2a, R′=CH_3 2b, R′=H

of fused (i.e., those with a bicyclo[3.3.0]octene skeleton) and bridged (i.e., those with a 7-alkylidenebicyclo[2.2.1]-heptane skeleton) cycloadducts **3–15**. To promote the encounter of diylophile and diyl and to minimize dimerization, the diazene was added via syringe pump to a refluxing solution of the diylophile in THF.

Table I

entry	diylophile		diyl	cycloadducts (isolated yields)	dimer (%)
1	PhCHO	2a		 3a, 33% β-Ph 3b, 4% α-Ph 4a, 37% β-Ph 4b, 4% α-Ph	15
2	$E_2C{=}O$ E=CO_2Et	2a	 5 (27%) 6 (19%) 7 (17%)		14
3	$E_2C{=}O$	2b	 8 (75%) [a]		b
4	$Ph_2C{=}S$	2a	 9 (50%) 10 (22%) 11 (19%)		4
5	PhN=CHPh	2a	 12a, 3% α-Ph 12b, 37% β-Ph 13 (35%)		8
6	$(CH_2O)_n$ $ZnCl_2$	2a	 14 (87%) [a]		b
7	EC≡CE E=CO_2CH_3	2a	 15 (76%)		b

[a] Regioismer and dimer not detected. [b] Dimer not detected.

Examination of Table I reveals several points worth noting. First, in all cases, fused cycloadducts are formed to a larger extent than are bridged. Second, in many instances dimerization occurred despite the use of syringe pump techniques. Third, of the diylophiles tested, thiobenzophenone proved to be the most reactive. To illustrate this point, a series of competition experiments were conducted, each involving equimolar amounts of a pair of diylophiles. From a run using benzaldehyde and thiobenzophenone, only thiobenzophenone-derived cycloadducts could be detected by capillary column GC analysis. Dimethyl fumarate proved to be a more successful competitor. Even so, approximately 4 to 5 times as much

(1) For a recent review concerning the use of heterodienophiles in Diels–Alder reactions, see: Weinreb, S. M.,; Staib, R. R. *Tetrahedron Report No. 136*, **1982**, *38*, 3087. For an up-to-date review of the intramolecular Diels–Alder reaction, see: (a) Fallis, A. G. *Can. J. Chem.* **1984**, *62*, 183–234. (b) Ciganek, E. In "Organic Reactions"; Wiley: New York, 1984; Vol. 32, Chapter 1.

(2) For some recent examples of the intramolecular 1,3-diyl trapping reaction, refer to: (a) Little, R. D.; Stone, K. J. *J. Am. Chem. Soc.* **1983**, *105*, 6976. (b) Little, R. D.; Highby, R. G.; Moeller, K. D. *J. Org. Chem.* **1983**, *48*, 3139. (c) Little, R. D.; Muller, G. W.; Venegas, M. G.; Carroll, G. L.; Bukhari, A.; Patton, L.; Stone, K. *Tetrahedron* **1981**, *37*, 4371. (d) Stone, K. J., Little, R. D. *J. Am. Chem. Soc.* **1985**, *107*, 2495–2505.

(3) Recently, Trost and Bonk reported an interesting [3 + 2]-type cycloaddition reaction between [2-(acetoxymethyl)-3-allyl]tri-*n*-butylstannane and both aldehydes and imines, leading to the production of heterocycles. See: Trost, B. M.; Bonk, P. J. *J. Am. Chem. Soc.* **1985**, *107*, 1778–1781.

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