Bu-2313, A NEW ANTIBIOTIC COMPLEX ACTIVE AGAINST ANAEROBES

II. STRUCTURE DETERMINATION OF Bu-2313 A AND B

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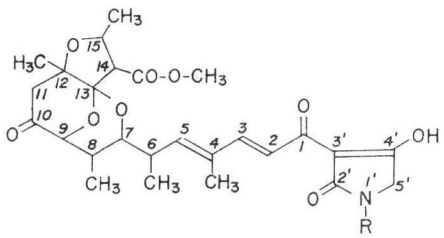
The structures of Bu-2313 A and B have been determined. They are dienoyltetramic acid-containing antibiotics structurally related to streptolydigin and tirandamycin.

Bu-2313 is a new antianaerobic antibiotic complex produced by the unidentified actinomycete strain No. E864-61. The production, isolation and various properties of Bu-2313 have been reported in the preceding paper[1]. Two components of the antibiotic complex, Bu-2313 A and B, have been isolated in crystalline form and showed properties similar to those of the tirandamycin and streptolydigin group of antibiotics. This paper presents evidence to show that Bu-2313 A (**I**) and B (**II**) possess the following structures:



Bu-2313 A (**I**) R=CH_3
Bu-2313 B (**II**) R=H

General Structural Characteristics

Bu-2313 A (**I**) and B (**II**) are acidic substances obtained as pale yellow crystals, both giving a positive reaction with ferric chloride. Their physico-chemical properties are summarized in Table 1. The molecular formula of $C_{27}H_{35}NO_9$ and $C_{26}H_{33}NO_9$ were assigned for **I** and **II**, respectively, based on their microanalyses and mass spectra.

The NMR spectrum of **I** showed three $\rangle$CH–$\underline{CH_3}$, two $\rangle$C–$\underline{CH_3}$, one N–$\underline{CH_3}$ and one COO$\underline{CH_3}$ signals, while **II** exhibited three $\rangle$CH–$\underline{CH_3}$, two $\rangle$C–$\underline{CH_3}$ and one COO$\underline{CH_3}$ (appeared as a 5H singlet combined with another 2H signal), but no N–CH_3 signal. Otherwise the NMR spectra of **I** and **II** are very similar, which, coupled with the assigned molecular formula, suggested that **I** is an N-methyl derivative of **II**.

The presence of a methyl ester group in **II** was confirmed by the hydrolysis of **II** in 1 N NaOH - CH_3OH (1: 1) at room temperature to afford acid **III** which analyzed as $C_{25}H_{31}NO_9$. **III** showed a UV absorption spectrum similar to that of **II**, indicating that the chromophore portion of **II** remained intact during alkaline hydrolysis. The O-methyl signal of **II** at around δ 3.76 disappeared in the NMR spectrum of **III**, leaving a 2H singlet at δ 3.74.

Table 1. Physicochemical properties of Bu-2313 A and B.

	Bu-2313 A	Bu-2313 B
Formula	$C_{27}H_{35}NO_9$	$C_{26}H_{33}NO_9$
Microanalysis	(C'd) (Fd)	(C'd) (Fd)
C:	62.65 62.57	62.03 61.77
H:	6.82 6.64	6.56 6.80
N:	2.71 2.60	2.78 2.65
M.p. (°C)	116~118	160~162
$[\alpha]_D^{26}$ in MeOH	−58°	−69.9°
pKa' in aq. EtOH	5.2	4.9
Titration equivalent	519	509
Mass, M^+ (*m/e*)	517	503

(C'd)=Calc'd, (Fd)=Found

Table 2. ^{1}H-NMR data of Bu-2313 A and B ($CDCl_3$, 60 MHz).

Assignment of protons	Chemical shift δ in ppm (J in Hz)	
	Bu-2313 A (I)	Bu-2313 B (II)
C-8 CH_3	0.86, d, (7.0)	0.87, d, (7.0)
C-6 CH_3	1.05, d, (7.0)	1.03, d, (7.0)
C-15 CH_3	1.32, d, (6.0)	1.31, d, (6.0)
C-12 CH_3	1.42, s	1.42, s
C-4 CH_3	1.90, s	1.87, s
H-8	2.0, m	2.07, m
H-11	2.55, d, (17.0)	2.56, d, (17.5)
H-6	2.8, m	2.76, m
H-14	2.91, d, (8.5)	2.92, d, (7.5)
H-11	2.98, d, (17.0)	2.95, d, (17.5)
1'-N-CH_3	3.03, s	—
H-7	3.42, dd, (11.0 and 2.0)	3.36, dd, (11.0 and 2.0)
H-5'	3.69, s	3.76, s
$COOCH_3$	3.78, s	3.76, s
H-9	3.99, d, (5.0)	4.01, d, (5.0)
H-15	4.48, d-q, (6.0 and 8.5)	4.49, d-q, (6.0 and 7.5)
H-5	5.97, br-d, (10.0)	5.96, br-d, (10.0)
H-2	6.98, d, (15.5)	6.96, d, (15.5)
1'-NH	—	6.98, br
H-3	7.42, d, (15.5)	7.41, d, (15.5)

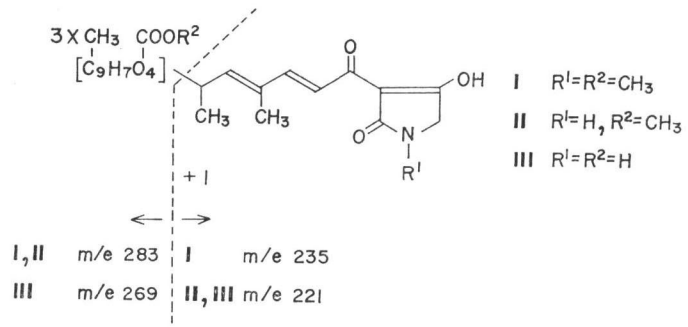
The UV spectrum of **I** in acidic solution exhibited absorption maxima at 242, 358, 375 nm ($E_{1cm}^{1\%}$ 170, 645, 555), and that of **II** at 237, 353, 370 nm ($E_{1cm}^{1\%}$ 170, 668, 580). In alkaline solution maxima occurred at 262, 286, 337 nm ($E_{1cm}^{1\%}$ 375, 393, 430) in **I** and at 253, 286, 331 nm ($E_{1cm}^{1\%}$ 345, 411, 467) in **II**. These hypsochromic shifts of UV absorption in alkaline solution observed for **I** and **II** are similar to those reported for tirandamycin[2] and streptolydigin[3] which contain dienoyltetramic acid chromophore in the molecule.

The presence of dienoic acid chromophore was supported by the NMR spectra of **I** and **II** (Table 2). The spectrum of **II** showed two low field sharp doublets (J=15.5 Hz) at δ 6.96 (H-2) and δ 7.41 (H-3), indicating a trans substitution of the α,β-double bond and no proton in the γ-position. The γ-methyl (C-4 CH_3) signal was found at δ 1.87 and the δ-proton (H-5) appeared at δ 5.96 as a doublet (J=10 Hz) broadened by coupling with the γ-methyl protons. The above-mentioned signals were also observed in the spectrum of **I**.

The NMR spectrum of **II** contained an amide NH at δ 6.98 which disappeared by D_2O addition. This NH signal was not present in the spectrum of **I**, which instead showed an N–CH_3 singlet at δ 3.03. A two-proton methylene singlet (H-5') was observed in the NMR of both **I** and **II**. The amide and methylene signals mentioned above were assigned to those of the tetramic acid moiety in comparison with the NMR spectrum of tirandamycin[4].

The mass spectrum of **II** showed the molecular ion peak at *m/e* 503 along with an intense peak at *m/e* 283 and a base ion peak at *m/e* 221. The *m/e* 221 peak was also observed with tirandamycin and assigned to the dienoyltetramic acid moiety. The mass spectrum of **I** showed the molecular ion peak at *m/e* 517 and a base peak at *m/e* 235, each being 14 mass units higher than those of **II**, while an in-

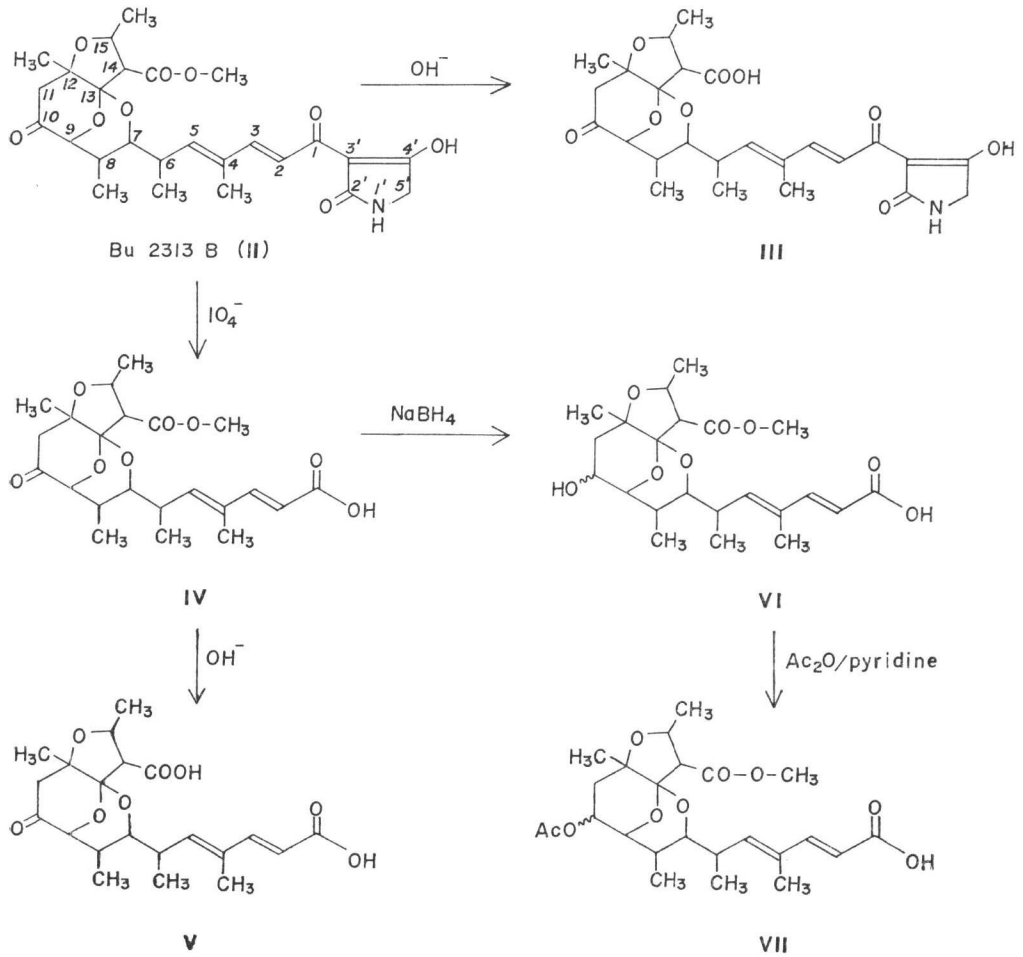
tense peak at *m/e* 283 was the same as that observed with **II**. In the mass spectrum of **III**, the base peak at *m/e* 221 was the same as that of **II**, while the molecular ion at *m/e* 489 and an intense peak at *m/e* 269 were 14 mass units lower than those of **II**. On the basis of the above-described spectral information, the following partial structures were given to **I**, **II** and **III**:



Chemical Transformation of II (Scheme 1)

Periodate oxidation of **II** under cooling afforded **IV** ($C_{22}H_{30}O_8$) which retained, as shown by NMR, three $\rangle$CH–CH$_3$ (δ in ppm: 0.87, 1.03 and 1.31), two $\rangle$C–CH$_3$ (1.42 and 1.79) and one COOCH$_3$

Scheme 1.



(3.76) groups. The IR spectrum of **IV** exhibited three absorption bands in the carbonyl region (1740, 1725 and 1680 cm^{-1}) and a hydrogen-bonded broad OH band centered at 2650 cm^{-1} characteristic to carboxylic acids.

The acid **IV** was hydrolyzed in alkaline methanol to give a dibasic acid **V** ($C_{21}H_{28}O_8$) whose IR and NMR spectra showed a lack of carbonyl band at 1740 cm^{-1} and O-methyl singlet at δ 3.76 as was the case observed in the conversion of **II** to **III**.

The UV spectrum of **IV** showed a maximum at 257 nm (ε 26,000) which is consistent with that for a dienoic acid as exemplified by tirandamycic acid (λ_{max} 260 nm, ε 27,400)[4] and streptolic acid (λ_{max} 261 nm, ε 28,200)[5]. This was also supported by the NMR spectrum of **IV** which showed an AB quartet of olefinic protons (δ 5.72 and 7.25 ppm) in a similar region to that reported for tirandamycic acid[4].

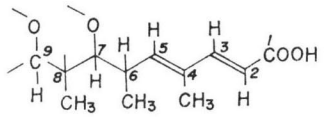
The partial structure *A* shown below was established for the C-1 to C-9 skeleton of **IV** by an NMR spin-decoupling experiment (Table 3). When a multiplet at around δ 2.76 (H-6) was irradiated, two doublets at δ 1.03 (C-6 CH_3) and at δ 5.89 (H-5) changed to two singlets and in addition a double doublet at δ 3.36 (H-7) collapsed to a doublet with J=11.0 Hz. The H-7 signal also changed to a doublet with J=2.0 Hz by irradiation at δ 2.07 (H-8). The same irradiation affected two doublets at δ 0.87 (C-8 CH_3) and δ 4.01 (H-9) to become singlets. The low chemical shifts of H-7 and H-9 suggested that carbons at C-7 and C-9 should be linked with an oxygen atom.

Table 3. Spin decoupling experiment on **IV**.

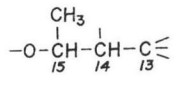
Proton (δ in ppm)						Multiplicity change	Splitting decoupled, Hz
Irradiated			Observed				
H-6		2.76	C-6	CH_3	1.03	d → s	7.0
			H-5		5.89	d → s	10.0
			H-7		3.36	dd → d	2.0
H-8		2.07	H-7		3.36	dd → d	11.0
			C-8	CH_3	0.87	d → s	7.0
			H-9		4.01	d → s	5.0
C-15	CH_3	1.31	H-15		4.49	dq → d	6.0
H-15		4.49	C-15	CH_3	1.31	d → s	6.0
			H-14		2.92	d → s	7.5
H-14		2.92	H-15		4.49	dq → q	7.5

The second structural unit *B* shown below was deduced also from the results of spin-decoupling experiment (Table 3). The irradiation of methyl doublet at δ 1.31 (C-15 CH_3) changed a low field methine signal (H-15, δ 4.49) from a double-quartet to a clear doublet. Furthermore, each of two doublets at δ 1.31 (C-15 CH_3) and δ 2.92 (H-14) collapsed to singlets by the irradiation at δ 4.49 (H-15), indicating that C-13 had no proton. The C-15, which bears a proton appearing at low field (δ 4.49), should be linked with an oxygen atom other than epoxide oxygen. A methine proton of epoxide ring should appear at around 3 ppm. Chemical shift of H-14 (δ 2.92) implies that C-14 links with an sp^2 carbon or forms an oxirane ring with C-13.

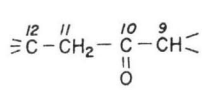
As shown above, a further three-carbon unit *C* adjacent to C-9 in the structure of **IV** was deduced from the NMR data of $NaBH_4$ reduction product **VI** ($C_{22}H_{32}O_8$) and its acetyl derivative **VII** (Scheme 1). The doublet H-9 methine signal of **IV** (δ 4.01) changed to a multiplet in **VI** (δ 3.90) and **VII** (δ 4.00). One proton multiplet newly appeared in the spectrum of **VI** at around δ 4.2, which shifted to



A

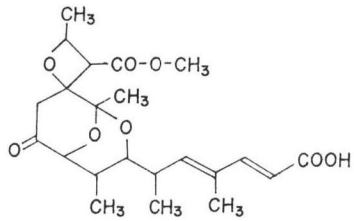


B

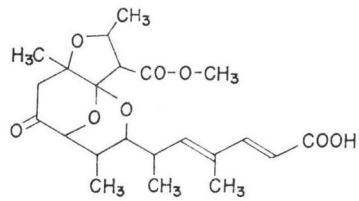


C

δ 5.25 in the O-acetate, **VII**. In addition, two doublets at δ 2.56 and δ 2.95 with a large J value (17.5 Hz), assigned to the isolated geminal methylene protons (H-11) of **IV**, collapsed to multiplets and shifted to around δ 1.8 in **VI** and δ 2.2 in **VII**. Combination of the structural units *A*, *B* and *C* together with a tertiary carbon-linked methyl and a methyl ester groups allows two possible structures, *D* and *E*, for compound **IV**.



D



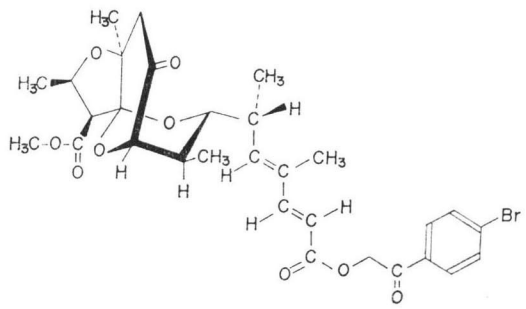
E

An X-ray crystallographic study was undertaken in order to determine whether **IV** has a spirooxetane structure (*D*) or a fused oxolane ring system (*E*).

X-Ray Structure of *p*-Bromophenacyl Ester of IV

The *p*-bromophenacyl ester of **IV** (**VIII**, $C_{30}H_{35}BrO_9$) was prepared and crystallized from ethyl acetate-methanol to afford colorless monoclinic crystals. The structure of **VIII** was determined by K. SASAKI[6] (Nagoya University) using a Hilger-Watts four-circle diffractometer, and the absolute configuration shown below was assigned to **VIII**.

The NMR spectra of **IV** and **VIII** are very similar and suggested that both compounds have the same stereochemistry in the fused ring structure and dienoyl moiety. Hence, the structure *E* shown ahead was assigned to **IV**, which in turn established the complete structures of Bu-2313 A (**I**) and B (**II**).

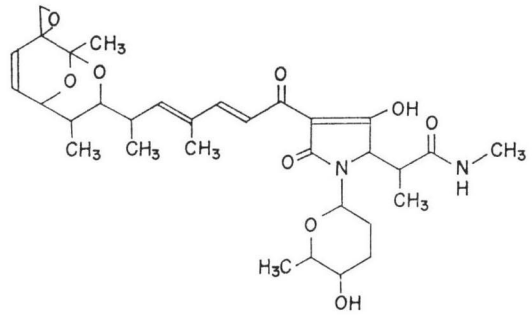


VIII

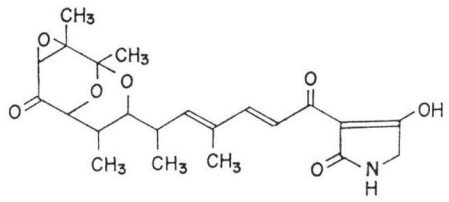
Discussion

Bu-2313 A and B are structurally related to streptolydigin and tirandamycin, all having the same conjugation system of dienoyltetramic acid as a chromophore. The presence of a tricyclic ketal structure is another common feature of these antibiotics. The structures of Bu-2313 A and B are unique in that they have a fused oxolane ring system instead of the oxirane (spiro or fused) ring of streptolydigin and tirandamycin.

After the present work had been completed, a new member of this family of antibiotics named nocamycin appeared in a Russian publication[7,8]. The molecular formula reported for nocamycin ($C_{26}H_{38}NO_9$) is coincident with that of Bu-2313 B and, in addition, the physico-chemical properties

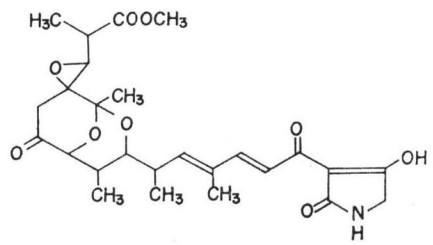


Streptolydigin



Tirandamycin

and spectral data of nocamycin are very close to those of Bu-2313 B. A recent publication[9] reported that nocamycin has a tricyclic ketal structure including a spiro oxirane ring as shown below. Whether Bu-2313 B and nocamycin are identical should await the results of direct comparison of the two antibiotics.



Nocamycin

Experimental

Alkaline hydrolysis of **II**

A solution of **II** (1.32 g) in 20 ml of 1 N NaOH - methanol (1: 1) was allowed to stand at room temperature for two days. The hydrolyzate was evaporated *in vacuo* and the residue taken up in chloroform. The solvent extract was washed by water, dried over Na_2SO_4 and concentrated *in vacuo*. The resultant solid was crystallized from ethyl ether to yield yellow crystals (**III**, 1.0 g). M.p. 223~224°C. λ_{max}^{EtOH} nm (ε): 235 (6,800), 280 (10,600), 352 (24,200).

Anal. Calc'd for $C_{25}H_{31}NO_9$: C 61.34, H 6.38, N 2.86.
Found: C 61.32, H 6.44, N 2.76.

Periodate oxidation of **II**

To a solution of **II** (5.03 g, 10 m mol) in 0.1% NaOH was added sodium periodate (12 g, 56 m mol) under vigorous stirring at 10°C. The reaction mixture was stirred for 2 days and then extracted with chloroform. The extract was dried over Na_2SO_4 and concentrated *in vacuo* to dryness. The solid thus obtained was crystallized from benzene - *n*-hexane to give **IV** (3.6 g; yield 64%). M.p. 130~132°C. λ_{max}^{EtOH} 257 nm (ε 26,000).

Anal. Calc'd for $C_{22}H_{30}O_8$: C 62.55, H 7.16.
Found: C 62.57, H 7.15.

Mass spectrum: *m/e* 423 ($M^+ + 1$).

Alkaline hydrolysis of **IV**

A solution of **IV** (211 mg) in 8 ml of 10% NaOH and 4 ml of methanol was allowed to stand overnight. After evaporation of methanol, the aqueous concentrate was adjusted to pH 1.0 by conc.HCl and extracted with chloroform. The chloroform extract was washed with water, dried over Na_2SO_4 and concentrated *in vacuo* to dryness. The residue was crystallized from a mixture of methylene chloride - ethyl ether to give **V** (80 mg). M.p. 239~240°C. λ_{max}^{EtOH} 259 nm (ε 25,100).

Anal. Calc'd for $C_{21}H_{28}O_8 \cdot \frac{3}{4}H_2O$: C 59.78, H 7.05.
Found: C 59.79, H 6.88.

Mass spectrum: *m/e* 408 (M^+).

Sodium borohydride reduction of **IV**

To a solution of **IV** (500 mg, 1.19 m mol) in 5 ml of methanol was added 100 mg (2 m mol) of sodium borohydride and the mixture allowed to stand overnight. The reaction mixture was concentrated

in vacuo and the concentrate treated with dil.HCl and extracted by chloroform. The extract was dried over Na_2SO_4 and concentrated *in vacuo* to dryness. The residue was crystallized from a mixture of ethyl ether and petroleum ether to give **VI** (450 mg; yield 90%). M.p. 217~218°C. λ_{max}^{EtOH} 258 nm (ε 24,400).

Anal. Calc'd for $C_{22}H_{32}O_8 \cdot \frac{1}{4}H_2O$: C 61.59, H 7.64.
Found: C 61.59, H 7.66.

Mass spectrum: *m/e* 425 ($M^+ + 1$).

Acetylation of **VI**

A solution of **VI** (222 mg) in 3 ml of dry pyridine was added to 1 ml of acetic anhydride and allowed to stand overnight. The mixture was poured onto ice water and then extracted with chloroform. The extract was washed with dil.HCl and water, dried over Na_2SO_4 and concentrated *in vacuo* to dryness. The resultant sticky solid was crystallized from a mixture of methylene chloride and petroleum ether to give **VII** (178 mg). M.p. 147~148°C. λ_{max}^{EtOH} 258 nm (ε 25,500).

Anal. Calc'd for $C_{24}H_{34}O_9$: C 61.79, H 7.34.
Found: C 61.55, H 7.52.

Mass spectrum: *m/e* 466 (M^+).

p-Bromophenacyl ester of **IV**

To a solution of **IV** (1.68 g, 2.77 m mol) in 40 ml of ethyl acetate were added *p*-bromophenacyl bromide (1.113 g, 3.04 m mol) and triethylamine (404 mg), and the mixture was allowed to stand overnight at room temperature. The reaction mixture was filtered and the filtrate was concentrated *in vacuo* to dryness. The resultant solid was crystallized from ethyl acetate-methanol to give **VIII** (2.01 g) which was recrystallized from the same solvent system to obtain monoclinic crystals for X-ray crystallographic analysis. M.p. 187~188°C. λ_{max}^{EtOH} 264 nm (ε 24,700).

Anal. Calc'd for $C_{30}H_{35}O_9Br$: C 58.16, H 5.69, Br 12.90.
Found: C 58.37, H 5.72, Br 12.65.

Acknowledgement

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