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Structure of Carzinophilin. IV.[1] Structure Elucidation by Nuclear Magnetic Resonance Spectroscopy. (2)

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The molecular formula of carzinophilin has been decided as $C_{31}H_{33}N_3O_{12}$. A structure for carzinophilin is proposed on the basis of the nuclear magnetic resonance data obtained.

Keywords——antibiotic; aziridine; 1,3-dioxane; oxazine; ^{1}H-NMR; spin decoupling; NOE; ^{13}C-NMR; gated decoupling; selective proton decoupling

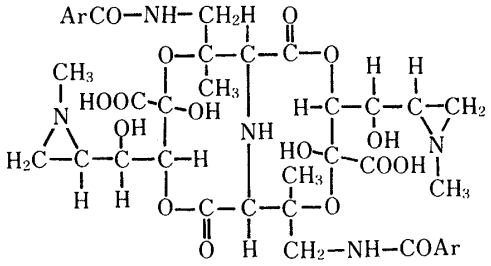
Carzinophilin (CZ, **1**) is an antitumor antibiotic isolated from *Streptomyces sahachiroi.*[2] Its molecular formula was given as $C_{60}H_{60}N_6O_{21}$,[3] which was later revised to $C_{50}H_{58}N_5O_{18}$[4] or $C_{33}H_{35}N_3O_{12}$.[5] These formulas were based on the molecular weights obtained by Rast's method using camphor as a solvent. It was suspected that these scattered results were unreliable and might have been influenced by the thermal instability and low solubility of CZ in camphor. For this reason, we previously reported the constituent compounds obtained by hydrolysis of CZ regardless of the molecular formula.[6] Nevertheless, Lown *et al.*[7] recently presented the structure (**1a**) for CZ by assuming the molecular formula to be $C_{50}H_{58}N_5O_{18}$ and by using the hydrolysis data obtained by us.[6] However, the molecular formula $C_{31}H_{33}N_3O_{12}$ has been obtained by mass spectrometry, so that **1a** seems very improbable. In a preliminary paper,[1] we proposed the structure (**1b**) (Chart 1) on the basis of nuclear magnetic resonance (NMR) spectroscopy. We now report in more detail the structure of CZ as revealed in further investigations by NMR spectroscopy.

CZ (**1**) is an acidic compound and afforded the neutral *p*-bromobenzoate (**2**) on treatment with *p*-bromobenzoyl chloride. Hydrogenation of **1** over a platinum catalyst in dioxane afforded acidic dihydro-CZ (**3**), which gives the neutral *p*-bromobenzoate (**4**).

The molecular weights of the compounds (**1**—**4**) could not be determined by conventional mass spectrometry. However, the molecular secondary ion mass spectrum (SIMS) of **4** using a glycerol matrix provided the precise molecular weight, corresponding to the molecular formula $C_{31}H_{33}N_3O_{12}$ for **1**. The ^{1}H- and ^{13}C-NMR spectra of **1** showed thirty-three protons including four protons exchangeable with deuterium oxide and thirty-one carbons, respectively, which are consistent with the molecular formula given above (Tables I and II). Protons were assigned by spin decoupling and nuclear Overhauser effect (NOE) experiments (see Experimental) as well as by comparison with the proton chemical shifts of related compounds. Carbon assignments were based on gated decoupling and selective proton decoupling experiments (Table II). These data indicated that **1** consists of three structural units (A, B and C).

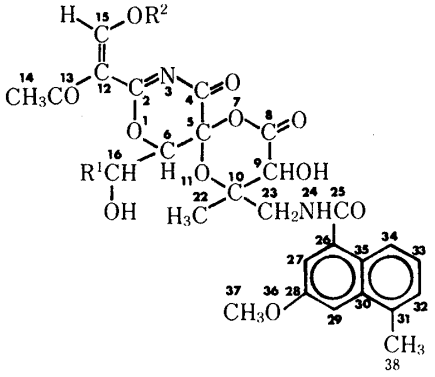
Unit (A)

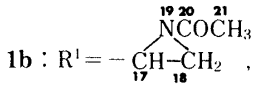
Protons and carbons of a 1,3,5-trisubstituted naphthalene ring and of two substituents (CH_3O and CH_3) were easily assigned. The third substituent was proved as follows. Selective

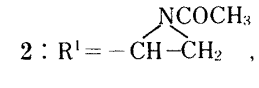


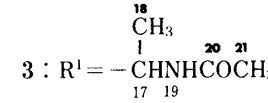
Ar = (3-CH_3O)(5-CH_3)$C_{10}H_5$-

1a



1b : R^1 =  , R^2 = H

2 : R^1 =  , R^2 = *p*-BrC_6H_4CO-

3 : R^1 =  , R^2 = H

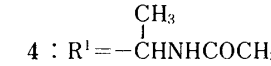
4 : R^1 =  , R^2 = *p*-BrC_6H_4CO-

Chart 1

(one-bond and long-range) proton decoupling experiments showed a methyl group (δ_H 1.53; δ_C 17.1 for C-22), a methylene group (δ_H 3.01 and 2.82; δ_C 53.8 for C-23), a methine group (δ_H 5.12; δ_C 76.7 for C-9) and two quanternary carbons (δ_C 164.3 for C-8; δ_C 56.2 for C-10) to arrange as depicted in Chart 2. A coupling (J_{CH} = 4 Hz) between 9-OH (δ_H 8.20, exchangeable with deuterium oxide) and C-8 was observed. Decoupling of 24(N)–H (δ_H *ca.* 7.35, exchangeable with deuterium oxide) sharpened the signals of C-10, C-23 and C-25. These observations distinguish between two protons exchangeable with deuterium oxide and demonstrate the existence of α-hydroxycarbonyl function in unit (A).

Unit (B)

Spin decoupling experiments showed the presence of a spin system composed of six protons, and coupling constants observed are as follows: J_{HH} = 4 Hz between 6-H (δ_H 5.50) and 16-H (δ_H 4.64), J_{HH} = 1 Hz between 6-H and 17-H (δ_H 3.39), J_{HH} = 6 Hz between 16-H and 17-H, J_{HH} = 2 Hz between 16-H and 16-OH (δ_H 3.82, exchangeable with deuterium oxide), J_{HH} = each 6 Hz between 17-H and 18-H_2 (δ_H 2.69 and 2.32, J_{gem} = 1 Hz). One-bond proton decoupling experiments correlated 6-H to C-6 (δ_C 84.4), 16-H to C-16 (δ_C 76.9) and 21-H_3 (δ_H 2.18) to C-21 (δ_C 20.8). The latter was distinguished from 14-H_3 by NOE experiments. Decoupling of 17-H (δ_C 46.6 for C-17) sharpened the signal of C-20 (δ_C 173.0). In addition, decoupling of 18-H (δ_H 2.32; δ_C 36.7 for C-18) and 21-H_3 changed the complex signal of C-20 to a doublet (J_{CH} = 2 Hz). On hydrogenation of **1** to **3**, the methylene group at the 18-position was converted into a methyl group (δ_H 1.24, d, J_{HH} = 5 Hz; δ_C 14.1, q), and the resonance of C-17 was shifted downfield by 11.5 ppm. These observations are consistent with a ring-opening of the aziridine ring to an amide. Thus, unit (B) was established as a 1,1-disubstituted 3,4-acetylimino-2-butanol function.

TABLE I. ^{1}H-NMR Data for **1**—**4**[a]

	1	**2**	**3**	**4**
6-H	5.50 dd (4, 1)	5.56 d (4)	5.54 s	5.48 s
9-H	5.12 s	5.12 s	5.31 s	5.23 s
14-H_3	2.29 s	2.43 s	2.21 s	2.36 s
15-H	*ca.* 7.35	8.33 s	*ca.* 7.35	8.24 s
15-OH[b]	12.16 s		13.12 s	
16-H	4.64 ddd (6, 4, 2)	4.66 dd (5, 4)	4.13—3.82	4.10—3.84
16-OH[b]	3.82 d (2)	Overlapping with OMe	3.60 br s	3.60 s
17-H	3.39 dq (1, 6)	3.30 t (5)	4.13—3.82	4.10—3.84
18-H_2	2.69 dd (6, 1)	2.59 dd (5, 1)		
	2.32 dd (6, 1)	2.31 dd (5, 1)		
18-H_3			1.24 d (5)	1.22 d (5)
21-H_3	2.18 s	2.22 s	2.09 s	2.15 s
22-H_3	1.53 s	1.52 s	1.61 s	1.57 s
23-H_2	3.01 d (5)	3.01 d (4)	3.08 d (4)	3.08 d (4)
	2.82 d (5)	2.83 d (4)	2.82 d (4)	2.73 d (4)
27-H	7.96 d (3)	7.94 d (3)	7.96 (3)	7.97 d (3)
29-H	7.49 d (3)	7.45 d (3)	7.46 d (3)	7.43—7.32
32-H 33-H	*ca.* 7.35	*ca.* 7.35	*ca.* 7.35	*ca.* 7.35
34-H	8.53 dd (8, 3)	8.53 dd (8, 3)	8.64 dd (8, 3)	8.60 dd (8, 3)
37-H_3	3.97 s	3.94 s	3.95 s	3.97 s
38-H_3	2.67 s	2.66 s	2.63 s	2.63 s
NH[b]	*ca.* 7.35 (24-H)	11.49 s	8.27 s	8.24 s
			8.07 s	7.68 s
		8.42 s	7.66 s	7.59 s
OH[b]	8.20 s (9-OH)			
Aromatic		7.97 d (9)		7.86 d (9)
H		7.54 d (9)		7.43—7.32

a) Chemical shifts; δ, ppm. Coupling constants; Hz (in parentheses).
b) On addition of deuterium oxide, these signals disappeared.

Unit (C)

Two carbons (C-14 and C-15) bearing protons were confirmed by one-bond proton decoupling experiments. Long-range proton decoupling experiments showed that 15-OH (δ_H 12.16, exchangeable with deuterium oxide) is coupled to C-15 (δ_C 151.0) ($J_{CH}=3$ Hz) and C-12 (δ_C 118.7) (sharpened). Couplings between 15-H (δ_H *ca.* 7.35) and C-2 (δ_C 165.9) ($J_{CH}=6$ Hz) and between 15-H and C-12 (sharpened) were observed. In addition, a coupling between 14-H_3 (δ_H 2.29; δ_C 24.3 for C-14) and C-12 (sharpened) was observed. A coupling ($J_{CH}=4$ Hz) observed between 14-H_3 and C-13 (δ_C 191.7) proved that they constitute an acetyl group. Conversion of **1** and **3** into **2** and **4** shifted the resonances of 15-H's downfield by *ca.* 1 ppm, respectively. Transformation of **3** to **4** shifted the resonance of C-15 upfield by 11.7 ppm. Thus, unit (C) was established as a formylacetone enol function.

Two quaternary carbons remained undecided. Decoupling of 6-H sharpened the signals of C-4 (δ_C 162.1) and C-5 (δ_C 119.3). In addition, couplings between 6-H and C-2 ($J_{CH}=4$ Hz) and between 16-H and C-5 (sharpened) were observed.

The most reasonable combination of units (A, B and C) with the remaining nitrogen (one) and oxygen (three) atoms leads to the structure (**1b**) for **1**, having a dihydro-4*H*-1,3-oxazine ring, to which a 1,3-dioxane ring is linked in a spiro form.

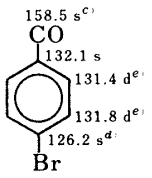
As previously reprorted,[6] hydrolysis of **1** with 5% ammonia at 80 °C for 30 min gave a

TABLE II. ^{13}C-NMR Data for **1**

C	δ (ppm)[a]	$^1J_{C,H}$ (Hz)	$^{>1}J_{C,H}$ (Hz)	Irradiated H	Resultant splitting
2	165.9 S dd		6, 4	6-H	d (6 Hz)
				15-H	d (4 Hz)
4	162.1 S br s			6-H	Sharpened
5	119.3 S br s			6-H	Sharpened
				16-H	
6	84.4 D m	156		6-H[b]	S
8	164.3 S m			9-H	d (4 Hz)
				9-OH	d (3 Hz)
9	76.7 D m	152		9-H[b]	S
				22-H_3	Sharpened
				23-H_2	br q (4 Hz)
10	56.2 S m			9-H	
				9-OH	Sharpened
				22-H_3	
				23-H_2	Quint (5 Hz)
				24-H	Sharpened
12	118.7 S m			14-H_3	
				15-H	Sharpened
				15-OH	
13	191.7 S q		4	14-H_3	s
14	24.3 Q s	130		14-H_3[b]	S
15	151.0 D d	184	3	15-H[b]	S
				15-OH	s
16	76.9 D m	152		16-H[b]	S
17	46.6 D m	180			
18	36.7 T m	178			
20	173.0 S m			17-H	Sharpened
				18-H[c] and 21-H_3	d (2 Hz)
21	20.8 Q s	130		21-H_3[b]	S
22	17.1 Q m	124		9-H	Sharpened
				22-H_3[b]	S
				23-H_2	d (3 Hz)
23	53.8 T m	172		22-H_3	Sharpened
				24-H	
25	156.1 S m			23-H_2	
				24-H	Sharpened
				27-H	
26	134.6 S dd		6, 4	24-H	d (6 Hz)
				34-H	d (4 Hz)
27	122.4 D d	160	6	27-H[b]	S
28	153.7 S q		4	37-H_3	s
29	108.5 D d	160	6	29-H[b]	S
30	128.2 S m			38-H_3	t (6 Hz)
31	133.4 S m			29-H and 33-H	q (3 Hz)
				38-H_3	t (6 Hz)
32	128.0 D m	160		32-H[b]	S
				38-H_3	d (6 Hz)
33	125.4 D s	160		33-H[b]	S
34	123.9 D d	160	6		
35	127.1 S m				
37	55.6 Q s	144			
38	20.1 Q d	124	4	32-H	s

a) Capital and small letters refer to one-bond and long-range couplings, respectively.
b) One-bond decouplings.
c) δ 2.32.

TABLE III. ^{13}C-NMR Data for **3** and **4**[a]

C	**3**[b]	**4**[b]
2	168.8 s	161.4 s
4	165.7 s	166.2 s
5	91.4 s	92.4 s
6	79.8 d	80.2 d
8	166.7 s	167.7 s
9	75.8 d	77.2 d
10	56.0 s	55.9 s
12	117.8 s	123.1 s
13	191.4 s	194.5 s
14	23.8 q	26.0 q
15	149.4 d	137.7 d
16	73.7 d	73.9 d
17	58.1 d	57.5 d
18	14.1 q	14.2 q
20	170.4 s	170.4 s
21	20.9 q	21.0 q
22	18.0 q	18.0 q
23	53.2 t	53.3 t
25	157.7 s	156.2 s[c]
26	134.3 s	133.0 s
27	122.1 d	122.5 d
28	155.8 s	155.4 s
29	108.5 d	108.8 d
30	128.1 s	129.0 s
31	133.1 s	132.1 s
32	127.7 d	127.7 d
33	125.2 d	125.2 d
34	124.0 d	123.4 d
35	126.9 s	126.8 s[d]
37	55.5 q	55.4 q
38	20.1 q	20.0 q
Others		

a) Chemical shifts; δ, ppm.
b) Small letters refer to the splitting observed in the off-resonance spectra.
c)—*e*) Assignments may be reversed.

precipitate, which affords (2*S*,3*S*)-4-amino-2,3-dihydroxy-3-methylbutanoic acid (**5**), 3-hydroxy-5-methylnaphthalene-1-carboxylic acid (**6**), glycine and an unidentified compound on further hydrolysis with 20% hydrochloric acid. The latter compound was identified as pyridine hydrochloride by direct comparison with an authentic sample. Furthermore, 3-methoxy-5-methylnaphthalene-1-carboxylic acid (**7**), its amide (**8**) and (2*S*,3*S*)-2,3-dihydroxy-4-(3-methoxy-5-methylnaphthalene-1-carboxamido)-3-methylbutanamide (**9**) were isolated from the turbid supernant produced by ammonolysis.

It is clear that the compounds (**5**—**9**) are formed from unit (A). Hydrolysis of the 7–8 bond and a retro-aldol cleavage of the 9–10 bond would give glycolic acid, which reacts with ammonia to yield glycine. The formation of glycine from glycolic acid under the same conditions as employed for the hydrolysis of **1** was confirmed. Since the formation of pyridine

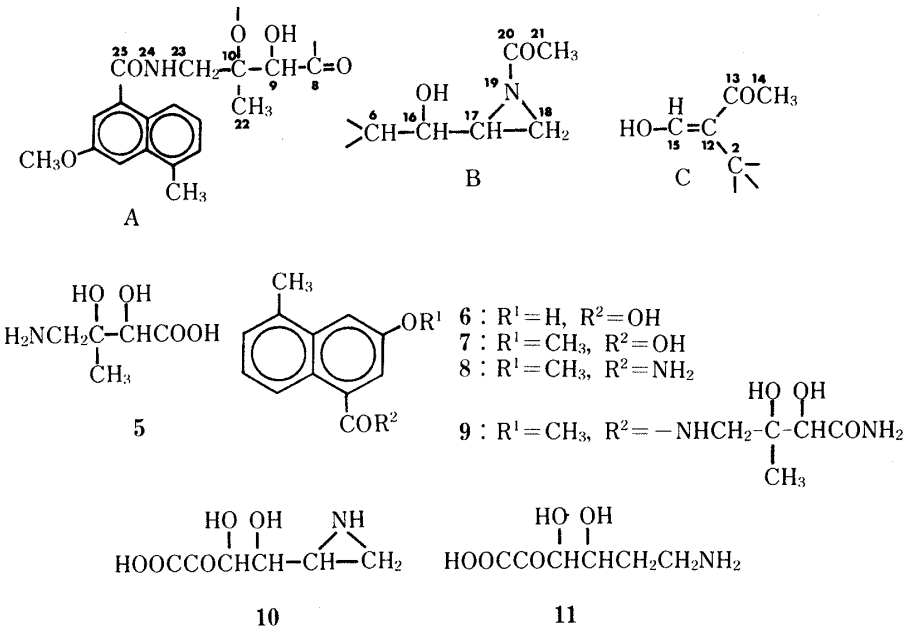


Chart 2

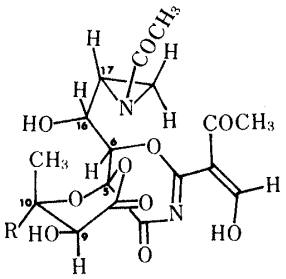
was also observed on hydrolysis of **1** with 20% hydrochloric acid, the nitrogen atom in the pyridine should not arise from ammonia. Hydrolysis of the 1–2, 3–4, 5–11, 7–8 and 19–20 bonds would afford 3,4-dihydroxy-5,6-imino-2-oxohexanoic acid (**10**). On the other hand, formic acid would be formed by a retro-Claisen cleavage of unit (C). Reduction of **10** with the formic acid to form 6-amino-3,4-dihydroxy-2-oxohexanoic acid (**11**), then ring closure between the carbonyl and amino groups, twice-dehydration and decarboxylation would account for the formation of pyridine.

Stereochemistry

It is likely that because of the presence of the gem-substituents at the 5- and 10-positions, the dioxane ring adopts a 1,4-twist form[8] with 22-H_3 (axial) as a stable conformation. Since the 9*S*,10*S*-configuration is assigned on the basis of the stereostructures of **5** and **9**, 9-OH is *cis* (equatorial) to 22-H_3. An NOE (5%) observed between 22-H_3 and 6-H suggests that the orientation of the 5–6 bond is *cis* to 22-H_3. Thus, the 5*S*-configuration can be assigned. A four-bond coupling (1 Hz) observed between 6-H and 17-H (*vide supra*) can be ascribed to an approximately W-shaped arrangement of these protons.[9] The 6–16 bond *trans* to the 5–7 bond generates severe steric hindrance between the aziridine and oxazine rings or the 10 eq-substituent in all possible forms of the oxazine ring. A chair form of the oxazine ring, in which the 6–16 bond is *cis* (equatorial) to the 5–7 bond, seems to be comparatively less hindered and would account for the NOE between 22-H_3 and 6-H.[10] As a result, the 6*R*-configuration can be assigned. Taking into account steric interaction between the aziridine and the oxygen (7) atom, the nitrogen (19) atom should be directed toward the oxygen (7) atom, and the 16–17 and 19–20 bonds should be *trans* with respect to the aziridine ring. Thus, the 17*S*-configuration can be assigned. In this geometrical arrangement, 18-H (δ_H 2.69) *syn* to the oxygen (1) atom would be more deshielded by the oxygen (1) atom[11] than 18-H (δ_H 2.32) *anti*.[12] Saturation of 14-H_3 caused 10 and 5% area increases in the signals of 6-H and 16-H, respectively.[13] These observations suggest that 14-H_3 is situated in the vicinity of the protons under consideration, so that the 2=3 and 12=15 bonds are *cis* with respect to the 2–12 bond, and the 16*S*-configuration can be assigned. In addition, an NOE (19%) observed between 14-H_3 and 15-H, which is due to rotation about the 12–13 bond, leads to the

Z-configuration for the 12=15 bond.

Consequently, the assumptions that (1) 6-H and 17-H are in an approximately W-shaped arrangement and (2) the molecule adopts less crowded geometry (not unreasonable) lead to the stereostructure (**1c**) (5*S*,6*R*,9*S*,10*S*,16*S*,17*S*) depicted in Chart 3. The infrared (IR) spectrum of **1** showed only an intramolecular hydrogen-bonding absorption (3540 cm^{-1}) for three hydroxyl groups and no free absorption. This hydrogen-bonding would contribute to the stabilization of the molecule as well as to the rigidity of the 6–16–17 bond (W-shaped arrangement). In order to confirm the structure of CZ, an X-ray analysis is to be undertaken in our laboratories.



R=(3-CH_3O)(5-CH_3)$C_{10}H_5CONHCH_2$-

1c

Chart 3

Experimental

Melting points were determined on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured with a JASCO DPI-181 polarimeter. Spectra were recorded on the following spectrometers: IR, Hitachi IR 260-30 (in chloroform unless otherwise noted); ^{1}H-NMR, JEOL JNM PS-100 (100 MHz), JEOL FX-100 (100 MHz) and Varian XL-100 (100 MHz) (in deuteriochloroform; tetramethylsilane); ^{13}C-NMR, JEOL JNM PFT-100 (25.2 MHz) (in deuteriochloroform; tetramethylsilane); molecular SIMS, Hitachi M-80A SIMS (glycerol matrix).

Carzinophilin (1)——Colorless granules of mp 215—230 °C (dec.) (chloroform/hexane). $[\alpha]_D^{19}$ +19.6° (c=0.9, chloroform). IR ν_{max} cm^{-1}: 3540 (ε=105) (OH···N and OH···O), 3345 (NH), 1731, 1708, 1651, 1623 (C=O) (c=3.13×10^{-4} mol/l, tetrachloromethane). ^{1}H-NMR NOE (%): 9-H→9-OH (7.5), 23-H (δ_H 3.01) (7.5); 9-OH→9-H (12.5), 23-H (δ_H 2.82) (7.5); 14-H_3→6-H (10), 15-H (19), 16-H (5); 15-H→14-H_3 (4.5); 16-H→17-H (8); 16-OH→6-H (11), 17-H (8); 17-H→16-H (5); 22-H_3→6-H (5); 23-H_2→9-H (6.5), 9-OH (6), 24-H (4); 32-H→38-H_3 (4.5); 37-H_3→27-H (6.5), 29-H (8.5); 38-H_3→29-H (14), 32-H (4.5). *Anal.* Calcd for $C_{31}H_{33}N_3O_{12}$: C, 58.21; H, 5.20; N, 6.56: for $C_{31}H_{33}N_3O_{12}\cdot 1/4H_2O$: C, 57.81; H, 5.24; N, 6.52. Found: C, 57.81; H, 5.34; N, 6.34.

Carzinophilin *p*-Bromobenzoate (2)——A solution of *p*-bromo-benzoyl chloride (17 mg) in anhydrous benzene (2 ml) was added to a mixture of **1** (20 mg) and K_2CO_3 (80 mg) in anhydrous benzene (5 ml). After being stirred at room temperature for 2 h, the reaction mixture was filtered and washed with benzene, then concentrated *in vacuo*. The residue was chromatographed on silica gel (2 g) with chloroform as the eluent to yield a crystalline solid (14 mg), which was recrystallized from chloroform/hexane to afford **3** (10 mg) as colorless needles of mp 169—170 °C. IR ν_{max} cm^{-1}: 3549 (OH), 3344 (NH), 1735, 1709, 1662, 1622 (C=O). *Anal.* Calcd for $C_{38}H_{36}BrN_3O_{13}$: C, 55.48; H, 4.41; Br, 9.71; N, 5.11. Found: C, 55.65; H, 4.11; Br, 9.60; N, 5.02.

Dihydrocarzinophilin (3)——A solution of **1** (10 mg) in anhydrous dioxane (2 ml) was hydrogenated over PtO_2 (5 mg) at room temperature for 3 h. The reaction mixture was filtered and washed with benzene. After removal of the solvent *in vacuo*, the residue was crystallized from chloroform/hexane to yield **2** (8 mg) as colorless granules of mp 128—130 °C. IR ν_{max} cm^{-1}: 3361 (NH and OH), 1731, 1709, 1655, 1622 (C=O).

Dihydrocarzinophilin *p*-Bromobenzoate (4)——A solution of *p*-bromobenzoyl chloride (6 mg) in anhydrous benzene (2 ml) was added to a mixture of **2** (8 mg) and K_2CO_3 (40 mg) in anhydrous benzene (5 ml), and the resulting mixture was stirred at room temperature for 1 h. Work-up by the same procedure as described above gave **4** (8 mg) as colorless plates of mp 125—127 °C (chloroform/hexane). IR ν_{max} cm^{-1}: 3512 (OH), 3384 (NH), 1728, 1709, 1660, 1620 (C=O). Molecular SIMS Calcd for $C_{38}H_{38}BrN_3O_{13}$: M, 823 and 825. Found m/z: $(M+Na)^+$, 846 and 848.

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