Biosynthetic Relationships in the Naphthocyclinone Series of Isochromane Quinone Antibiotics[1]

Bernd Krone,† Axel Zeeck,† and Heinz G. Floss*[2]

Organisch-Chemisches Institut der Universität, D-34 Göttingen, West Germany, and Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, Indiana 47907

Received February 2, 1982

The biosynthetic reaction sequence γ-naphthocyclinone (3) → β-naphthocyclinone (4) → β-naphthocyclinone epoxide (5) → α-naphthocyclinone (1) → α-naphthocyclinone acid (2) was demonstrated in *Streptomyces arenae* strain Tü 495 by feeding the strain ^{14}C-labeled naphthocyclinones prepared from [1-^{14}C]acetate. The monomeric hydroxyquinone 8, but not the corresponding dimer 9, may be an intermediate in naphthocyclinone formation.

The naphthocyclinones[3-6] are a subgroup of the family of isochromane quinone antibiotics, whose chemistry[7] and biosynthesis[8] have been the subject of recent reviews. Naphthocyclinones are unsymmetrical dimers represented by structures 1–7 (Chart I). The biosynthetic origin of α-naphthocyclinone from acetate/malonate via the polyketide pathway as shown in Scheme I has been demonstrated.[9] The present paper reports results which shed some light on the biosynthetic interrelationships among the naphthocyclinones.

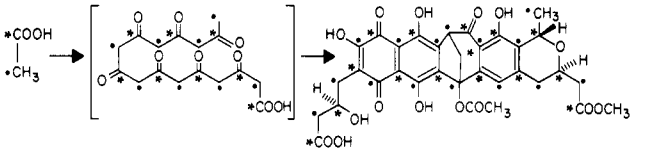
Results

To study interconversions of naphthocyclinones, we prepared these compounds in radioactively labeled form by feeding sodium [1-^{14}C]acetate to cultures of the naphthocyclinone-producing strain *Streptomyces arenae* Tü 495. The products were extracted from the cultures and separated by column chromatography. The individual naphthocyclinones were then further purified by preparative layer chromatography. The results are summarized in Table I. The majority of the labeled α-naphthocyclinone and α-naphthocyclinone acid were combined and degraded by established procedures[3,4] to the monomer 8, an aliquot of which was further oxidized[10] to the corresponding dimer 9.

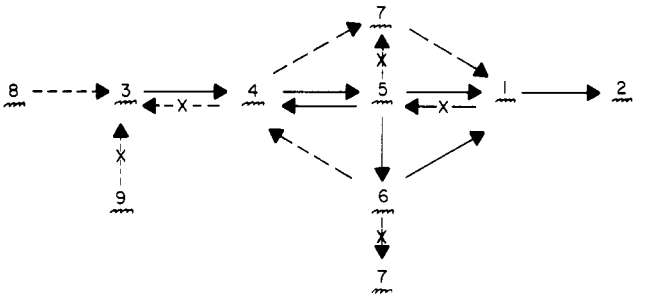
The labeled naphthocyclinones and compounds 8 and 9 were individually fed each to a 48-h-old 100-mL culture of *S. arenae* strain Tü 495. Except in the experiments with 8 and 9, which were harvested entirely after 24 h, a sample of 15 mL was drawn from each culture after 6 h, and the remaining 85 mL were harvested 24 h after precursor addition. Extraction gave the crude naphthocyclinone fractions, which contained between 35% and 55% of the administered radioactivity (Table II). Hence, in each case a substantial fraction of the administered precursor had been metabolized to nonextractable materials. The defatted naphthocyclinone fractions were further resolved by chromatography on 5 × 20 cm thin-layer plates. The radioactivity distribution between the individual naphthocyclinones was estimated by scanning the plates in a radiochromatogram scanner. All the visibly detectible naphthocyclinone bands were then separately scraped from the plates and their radioactivity determined by liquid scintillation counting. In a number of experiments some of the naphthocyclinone bands were not visible, and in all those cases there was also no corresponding radioactivity peak in the radiochromatogram. The results are summarized in Table III.

The radiochromatogram scans from the experiment with monomer 8 showed radioactivity matching the bands of 1, 2, 4, and 5, as well as unreacted 8, whereas in the experiment with 9 the unreacted precursor was the major labeled constituent, and no distinct zones were seen for the various naphthocyclinones. 1 and 2 derived from 8 were isolated by preparative layer chromatography, combined (15.2 mg), and converted into α-naphthocyclinone methyl ester methyl ether[3] which was rechromatographed (5.1 mg). Radioactivity analysis of the latter indicated an incorporation rate of 0.94% and a dilution factor[11] of 240. Similar treatment of 1 and 2 from dimer 9 gave an incorporation rate of 0.23% and a dilution factor of 820.

Scheme I. Biosynthetic Origin of α-Naphthocyclinone



Scheme II. Biosynthetic Interconversions of Naphthocyclinones[a]



[a] Conversions firmly established (solid arrow) or excluded (solid arrow with an X); conversions probably occurring (dashed arrow) or not occurring (dashed arrow with an X).

Discussion

The results summarized in Table III clearly establish the biosynthetic reaction sequence γ-naphthocyclinone (3)

(1) This work was supported by grants from the U.S. Public Health Service (AI 11728) and from NATO (RG 263.80).

(2) To whom reprint requests should be addressed at Department of Chemistry, Ohio State University, Columbus, OH 43210.

(3) Zeeck, A.; Mardin, M. *Justus Liebigs Ann. Chem.* **1974**, 1063.

(4) Zeeck, A.; Zähner, H.; Mardin, M. *Justus Liebigs Ann. Chem.* **1974**, 1100.

(5) Krone, B. Ph.D. Dissertation, University of Göttingen, 1979.

(6) Krone, B.; Zeeck, A., to be submitted for publication in *Justus Liebigs Ann. Chem.*

(7) Zeeck, A.; Krone, B. to be submitted for publication in *Prog. Chem. Org. Nat. Prod.*

(8) Floss, H. G. In "Antibiotics IV. Biosynthesis"; Corcoran, J. W., Ed.; Springer-Verlag: West Berlin, 1981; pp 215–235.

(9) Schröder, K.; Floss, H. G. *J. Org. Chem.* **1978**, *43*, 1438.

(10) Laatsch, H. *Justus Liebigs Ann. Chem.* **1980**, 1321. Laatsch, H., unpublished results.

(11) Incorporation rate = radioactivity of product × 100/radioactivity of precursor [%]. Dilution factor = specific molar radioactivity of precursor/specific molar radioactivity of product.

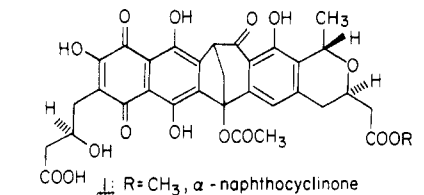
† Organisch-Chemisches Institut der Universität Göttingen.
* Purdue University.

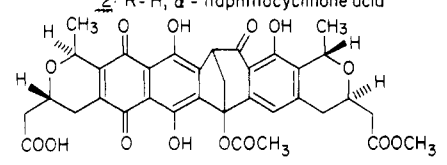
0022-3263/82/1947-4721$01.25/0

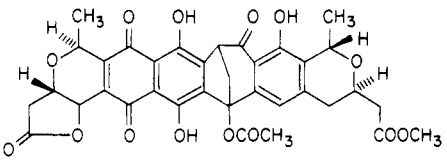
Table I. Preparation of Labeled Naphthocyclinones by Feeding Sodium [1-^{14}C]Acetate (3.19×10^9 dpm, 1.9×10^{10} dpm/mmol) to Cultures (Nine Flasks, 990 mL) of *S. arenae* Strain TU 495

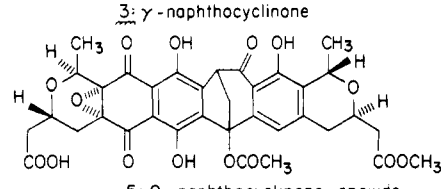
product	quantity obtained, mg	specific radioactivity, dpm/mmol	total radioactivity, dpm
α-naphthocyclinone (1)	31	9.9×10^7	4.6×10^6
α-naphthocyclinone acid (2)	71	11.4×10^7	9.2×10^6
β-naphthocyclinone (4)	1.5	5.0×10^7	1.2×10^5
β-naphthocyclinone epoxide (5)	6.8	3.2×10^7	3.1×10^5
β-naphthocyclinone chlorohydrin (6)	2.9	2.8×10^7	1.1×10^5
γ-naphthocyclinone (3)	0.6	5.6×10^7	5×10^4
δ-naphthocyclinone (7)	3.5	2.1×10^7	1.0×10^5

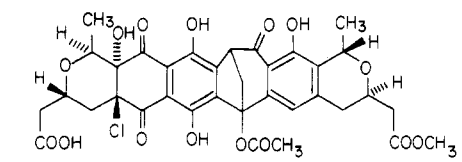
Chart I

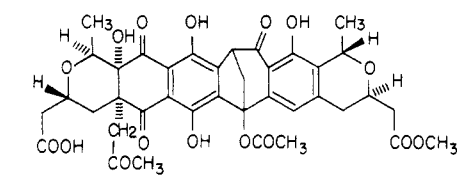


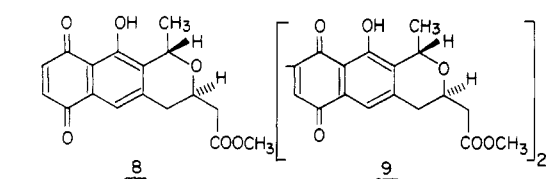






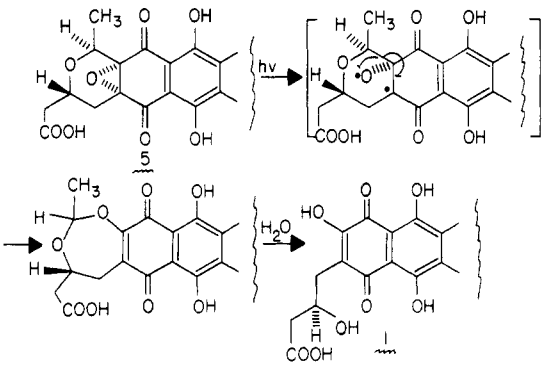






→ β-naphthocyclinone (4) → β-naphthocyclinone epoxide (5) → α-naphthocyclinone (1) → α-naphthocyclinone acid (2) as shown in Scheme II. 1 and 2 are clearly the end products in the pathway; they are not converted into any

Scheme III. Proposed Mechanism for the Photolytic Conversion of β-Naphthocyclinone Epoxide to α-Naphthocyclinone



of the other compounds. 3 is converted into all the other naphthocyclinones but is formed from none of them, placing it at the beginning of the reaction sequence. The conversion sequence 3 → 4 → 5 parallels the sequence of steps established by Omura and co-workers[12] in the nanaomycin series, where the lactone nanaomycin D is reduced[13] to nanaomycin A, which is then epoxidized[14] to nanaomycin E. A different situation may exist in the granaticin series, where the appearance of the lactone granaticin in the cultures seems to be preceded by that of the open-chain dihydrogranaticin,[15] and the enzymatic conversion of dihydrogranaticin to granaticin has been demonstrated.[16]

α-Naphthocyclinone is evidently formed by extrusion of a two-carbon unit, probably at the stage of β-naphthocyclinone epoxide. Chemical conversion into 1 has been observed upon photolysis of solutions of 5, and the mechanism shown in Scheme III has been proposed for this transformation.[5,6] It seems likely that a similar mechanism accounts for the enzymatic conversion. The chlorohydrin 6 is formed from the epoxide 5 and can be converted to 1, possibly by a direct route not involving 5 or 4 as intermediates. This again has a nonenzymatic parallel; 6 can also be photolyzed to give 1.[5,6] Similarly, δ-naphthocyclinone (7) seems to be formed from 4, although the mechanism of this conversion is obscure, and it is converted to 1 and 2 by a direct enzymatic route, apparently not via 4 or 5. On the other hand, it is possible that the conversion of 6 and 7 to 1 proceeds via 4 and/or 5 but that the stationary concentration of these intermediates is very low. In any case, it is apparent that the routes from 4 to

(12) Kitao, C.; Tanaka, H.; Minami, S.; Omura, S. *J. Antibiot.* **1980**, *33*, 711.
(13) Omura, S.; Tanaka, H.; Minami, S.; Takahashi, T. *J. Biochem. (Tokyo)* **1981**, *90*, 355.
(14) Omura, S.; Minami, S.; Tanaka, H. *J. Biochem. (Tokyo)* **1981**, *90*, 291.
(15) St. Pyrek, J.; Achmatowicz, O., Jr.; Zamojski, A. *Tetrahedron* **1977**, *33*, 673.
(16) Snipes, C. E.; Chang, C.-J.; Floss, H. G. *J. Am. Chem. Soc.* **1979**, *101*, 701.

Table II. Feeding Experiments with Labeled Naphthocyclinones and Their Potential Precursors in *S. arenae* Strain TU 495

compd fed	radioactivity fed, dpm	radioactivity recovered in crude naphthocyclinone fraction, dpm		
		after 6 h (15 mL)	after 24 h (85 mL)	total
α-naphthocyclinone (1)	1.2×10^6	4.1×10^4	4.1×10^5	4.5×10^5
β-naphthocyclinone (4)	1.2×10^5	1.1×10^4	5.2×10^4	6.3×10^4
β-naphthocyclinone epoxide (5)	3.1×10^5	3.0×10^4	1.5×10^5	1.8×10^5
β-naphthocyclinone chlorohydrin (6)	1.1×10^5	8.1×10^3	5.2×10^4	6.0×10^4
γ-naphthocyclinone (3)	5.0×10^4	4.2×10^3	1.9×10^4	2.3×10^4
δ-naphthocyclinone (7)	1.0×10^5	6.4×10^3	3.0×10^4	3.6×10^4
monomer 8	7.3×10^5			3.3×10^5
dimer 9	4.4×10^5			1.6×10^5

Table III. Radioactivity Distribution in the Naphthocyclinone Fraction after Feeding Specific Labeled Naphthocyclinones

compd fed	time, h	total radioactivity recovered in compound,[a,c] dpm (fraction of total radioactivity in purified naphthocyclinones/minimum incorporation rate)[b]						
		α-naphthocyclinone (1)	α-naphthocyclinone acid (2)	β-naphthocyclinone (4)	β-naphthocyclinone epoxide (5)	β-naphthocyclinone chlorohydrin (6)	γ-naphthocyclinone (3)	δ-naphthocyclinone (7)
α-naphthocyclinone (1)	6	140000 (91/11.7)	14000 (9/1.2)	ND	ND	ND	ND	ND
α-naphthocyclinone (1)	24	43000 (18/3.6)	197000 (82/16.4)	172	150	285	174	ND
β-naphthocyclinone (4)	6	11600 (49/9.7)	ND	7300 (30/6.1)	4100 (17/3.4)	ND	ND	870 (4/0.7)
β-naphthocyclinone (4)	24	3000 (35/3.7)	4400 (24/2.5)	3700 (29/3.1)	1500 (12/1.3)	ND	ND	ND
β-naphthocyclinone epoxide (5)	6	33000 (48/10.6)	5700 (8/1.8)	22000 (32/7.1)	ND	8000 (12/2.6)	ND	ND
β-naphthocyclinone epoxide (5)	24	4700 (9/1.5)	34000 (67/11.0)	3500 (7/1.1)	4300 (9/1.4)	3900 (8/1.3)	ND	ND
β-naphthocyclinone chlorohydrin (6)	6	2070 (33/1.9)	ND	870 (14/0.8)	ND	3300 (53/3)	ND	ND
β-naphthocyclinone chlorohydrin (6)	24	2500 (28/2.3)	1680 (19/1.5)	710 (8/0.6)	ND	2060 (45/3.7)	ND	ND
γ-naphthocyclinone (3)	6	1750 (37/3.5)	ND	2970 (63/5.9)	ND	ND	ND	ND
γ-naphthocyclinone (3)	24	2420 (40/4.8)	1300 (22/2.6)	700 (12/1.4)	650 (11/1.3)	280 (5/0.6)	26 0 (4/0.5)	370 (6/0.7)
δ-naphthocyclinone (7)	6	1560 (39/1.6)	ND	ND	ND	ND	ND	2470 (61/2.5)
δ-naphthocyclinone (7)	24	2300 (33/2.3)	1930 (28/1.9)	60	60	ND	ND	2620 (38/2.6)

[a] Normalized to a 100-mL culture. [b] Values in parentheses are given in percent. [c] ND = not determined.

1 via 6 or 7 are quantitatively much less significant compared to the main pathway 4 → 5 → 1, and in view of the low incorporation rates and the fact that the products were neither recrystallized to constant specific radioactivity nor degraded, these minor routes must be considered tentative. One rather unexpected transformation which seems to occur quite efficiently is that of 5 back into 4; such a conversion is somewhat unusual in a biological system. It may be noted that all the biosynthetic transformations established in this series have also been achieved chemically,[5,6] with the exception of the formation of 7 which has so far not been accomplished by chemical means.[5]

The role of monomer 8 and dimer 9 in the biosynthesis of naphthocyclinones is not entirely elucidated by our experiments. The incorporation rate of 8 into 1 plus 2 of almost 1% compares favorably with the values of 6–12% seen in the conversions of 4 and 5 to 1 plus 2, considering that the latter involve only one or two steps, suggesting that 8 may indeed be an intermediate in the biosynthesis. The fourfold lower incorporation of dimer 9, which should be a more proximate precursor, on the other hand, indicates that this compound is probably not on the biosynthetic pathway. Whether 8 labels both halves of the naphthocyclinone molecule or only one side could not be established due to lack of material. The best interpretation of the limited data available is that the biosynthesis proceeds through 8, but that dimerization occurs either at the stage of a product derived from 8 or involves 8 and another monomeric unit to give an unsymmetrical dimer. However, more experiments are needed to unequivocally define the role of 8 in naphthocyclinone biosynthesis.

Experimental Section

Materials and General Methods. [1-^{14}C]Acetate (specific activity 8.8 mCi/mmol) was purchased from ICN Pharmaceuticals. *Streptomyces arenae* strain Tü 495 was obtained from Professor Zähner, Tübingen, and was maintained and cultured as described earlier.[9] Thin-layer chromatography was carried out on 0.25-mm-thick 5 × 20 cm or 1-mm-thick 20 × 20 cm precoated silica plates (Brinkmann) which had been sprayed with 0.5 N L-(+)-tartaric acid solution. Quantitation of naphthocyclinones involved measurement of their absorption in the visible region in a Gilford Model 250 spectrophotometer. Radioactivity determinations were carried out on a Beckman LS 7500 scintillation counter by using [α-^{14}C]toluene as an internal standard. Radioactivity on chromatograms was located by using a Packard Model 7201 radiochromatogram scanner.

Preparation of Labeled Naphthocyclinones and Precursors. Sodium [1-^{14}C]acetate (1.45 mCi) was fed to nine cultures of *S. arenae* Tü 495, each containing 110 mL of medium in a 500-mL baffled flask, at 52 h after inoculation. The cultures were harvested 36 h later, and the naphthocyclinones were extracted from the culture medium and mycelium as described earlier[9] to give 168 mg of crude material (1.5 × 10^7 dpm, 0.48% incorporation). The crude naphthocyclinones were chromatographed on a column of 28 g of oxalic acid treated silica gel.[3] The column was developed first with chloroform/acetone (9:1), followed by chloroform/ethyl acetate/acetone (9:9:2). The eluates with the first solvent were pooled into three fractions containing, in that order, γ-naphthocyclinone, β-naphthocyclinone plus β-naphthocyclinone epoxide, and β-naphthocyclinone chlorohydrin plus δ-naphthocyclinone. The second solvent gave two fractions, the first containing α-naphthocyclinone and the second α-naphthocyclinone plus α-naphthocyclinone acid. These fractions were further resolved into the individual components by thin-layer chromatography with benzene/ether/acetone (9:9:2) as the solvent.

The majority of the α-naphthocyclinone and all of the α-naphthocyclinone acid were combined (about 100 mg) and dissolved in 30 mL of 2 N NaOH. After 30 min at room temperature, the solution was acidified to pH 3 with 1 N HCl, and 50 min later, the precipitate was collected by filtration, washed with water, and dried. The deacetyl-α-naphthocyclinone acid thus obtained was dissolved in 50 mL of 70% sulfuric acid, and the solution was kept at room temperature for 20 h and then poured into 1 L of ice–water. Extraction with ethyl acetate or filtration followed by extraction of the aqueous solution with ethyl acetate gave deacetylanhydro-α-naphthocyclinone acid. This compound then served as the starting material for the preparation of the diazomethane adduct and its further conversion by established procedures into compound 8[3] and the corresponding dimer 9.[10] The final purification of both of these compounds was achieved by thin-layer chromatography in the system benzene/ether/acetone (9:9:2).

Feeding of Labeled Naphthocyclinone Precursors. The incorporation of precursors and the interconversion of labeled naphthocyclinones was studied by feeding each compound to a 100-mL culture at 48 h after inoculation. An aliquot (15 mL) of the culture was harvested 6 h later and the remaining 85 mL 24 h after addition of the precursor. The cultures were worked up as described previously[9] to give a crude extract, which was further processed by repeated trituration with petroleum ether. In some cases, it was necessary to further treat the material by dissolving it in a minimal amount of chloroform (0.1–1 mL), adding this solution dropwise with stirring to 50 mL of hexane, and collecting the precipitate after 20 min. The resulting solid material was then suitable for chromatographic separation on tartaric acid treated thin-layer plates in the system benzene/ether/acetone (9:9:2) to give α-naphthocyclinone acid (R_f 0.18), α-naphthocyclinone (R_f 0.25), δ-naphthocyclinone (R_f 0.27), β-naphthocyclinone chlorohydrin (R_f 0.38), γ-naphthocyclinone (R_f 0.41), β-naphthocyclinone epoxide (R_f 0.44), and β-naphthocyclinone (R_f 0.48). The individual chromatograms were scanned for radioactivity. Following that, the visible naphthocyclinone bands were scraped off, placed in scintillation vials, and triturated with methanol (1 mL), followed by addition of scintillator solution 10 min later. The samples were then counted for radioactivity.

In the experiments with monomer 8 and dimer 9, the α-naphthocyclinone and α-naphthocyclinone acid were isolated by preparative layer chromatography, eluted separately, and then combined and converted into α-naphthocyclinone methyl ester methyl ether as described previously.[3] This material was further purified by repeated thin-layer chromatography by using the solvent system 9:1 chloroform/acetone (R_f 0.6).

Registry No. 1, 54826-93-6; 2, 54367-38-3; 3, 55095-58-4; 4, 55050-83-4; 5, 83333-54-4; 6, 83314-00-5; 7, 83333-55-5; 8, 83376-30-1.