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Isocochliodinol and Neocochliodinol, Bis(3-indolyl)-benzoquinones from *Chaetomium* spp.

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Two kinds of bis(3-indolyl)-dihydroxybenzoquinones, isocochliodinol and neocochliodinol, were isolated from *Chaetomium murorum* and *C. amygdalisporum*, respectively, and their structures were determined.

Keywords——isocochliodinol; neocochliodinol; mollicellin G; indolylbenzoquinones; *Chaetomium murorum*; *Chaetomium amygdalisporum*; cytotoxic agent

Since the discovery of chaetoglobosins, novel cytochalasans, surveys on mycotoxin production by *Chaetomium* spp. and related fungi have been conducted and the production of a number of potentially significant mycotoxins, such as sterigmatocystin, *O*-methylsterigmatocystin, chaetocin, chetomin, and chaetochromin, was found.[1] In the course of these studies, production of cochliodinol (2′,5′-dihydroxy-3′,6′-bis(5-(3-methyl-2-butenyl)-3-indolyl)-benzoquinone, **1**), isolated from *C. cochliodes* PALLISER and *C. globosum* KUNZE ex FRIES,[2] or an analog was recognized in cultures of *C. elatum* KUNZE ex FR., *C. murorum* CORDA, and *C. amygdalisporum* UDAGAWA & MUROI as a cytotoxic agent.[1b] Chromatographic separation of the dichloromethane extracts of the molds on rice culture revealed that the pigment from *C. elatum* was identical with cochliodinol (**1**), but the pigments from *C. murorum* and *C. amygdalisporum* were new isomers, named isocochliodinol (**2a**) and neocochliodinol (**3a**),[1b] respectively. This paper describes the structural elucidation of the two pigments.

The two new isomers have the same molecular formula, $C_{32}H_{30}N_2O_4$ (by mass spectroscopy (MS), field desorption (FD) mode), as cochliodinol. They each formed a diacetate (**2b**, **3b**). The similarity of the ultraviolet (UV) and infrared (IR) spectra of the isomers to those of cochliodinol (**1**) indicated the same nucleus, 2′,5′-dihydroxy-3′,6′-bisindolylbenzoquinone (Table I, Chart 1). The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectra (Table II) clearly disclosed the presence of 3-methyl-2-butenyl groups, symmetrically substituted on the indolyl groups. Thus, the two new members must be positional isomers of the prenyl groups of cochliodinol (**1**).

Comparison of the chemical shifts and the coupling patterns in the ^{1}H-NMR of the three compounds (Table II) showed some difference in the aromatic protons at the 4—7 positions of the indole ring. A nuclear Overhauser effect (NOE) study on **1** was effective for the establishing of the positions of the prenyl groups.[2c] Reexamination of the compound gave the following results (irradiated protons in parentheses); (NH) H-2 (δ 7.51) 26.2%, H-7 (δ 7.19) 6.5%, H-4 (δ 7.39) 0%; (H-8, δ 3.40) H-4 22.3%, H-6 (δ 6.90) 6.7%, H-7 0%; (H-2) H-6 0%. A similar study was carried out for isocochliodinol (**2a**) and the following results were obtained: (NH) H-2 (δ 7.39) 21.1%, H-7 (δ 7.01) 12.5%, H-4 (δ 7.38) 0%; (H-8, δ 3.32) H-7 12.5%, H-5 (δ 6.73) 13.1%, H-4 0%. These observations along with the coupling patterns shown in Table II indicated that the prenyl groups are at the 6-position of the indole rings. Thus, the structure of isocochliodinol was shown to be 2′,5′-dihydroxy-3′,6′-bis(6-(3-methyl-2-butenyl)-3-

TABLE I. IR and UV Data for Cochliodinol and Related Compounds

	IR ν_{max}^{KBr} cm^{-1}	UV λ_{max}^{EtOH} nm (log ε)
Cochliodinol (**1**)	3400, 3303, 1620, 1521, 1420, 1325, 1270, 1100, 1020, 983, 808, 780	280, 471 (4.49, 3.65)
Isocochliodinol (**2a**)	3410, 3351, 1603, 1530, 1442, 1330, 1277, 1103, 1040, 980, 818, 755	290, 470 (4.48, 3.63)
Isocochliodinol diacetate (**2b**)	3400, 3300, 1779, 1650, 1595, 1509, 1440, 1400, 1360, 1260, 1230, 1165, 1130, 1024, 890, 810, 745	288, 510 (4.48, 3.85)
Neocochliodinol (**3a**)	3400, 3352, 1604, 1600, 1525, 1430, 1330, 1272, 1110, 982, 795, 750	290, 470 (4.49, 3.60)
Neocochliodinol diacetate (**3b**)	3385, 2901, 1770, 1650, 1590, 1509, 1425, 1360, 1239, 1155, 1120, 1060, 1010, 870, 808, 783, 748	290, 498 (4.48, 3.85)
Neocochliodinol dimethyl ether (**3c**)	3298, 1640, 1590, 1518, 1424, 1280, 1240, 1117, 1074, 1025, 782, 750	290, 480 (4.48, 3.58)

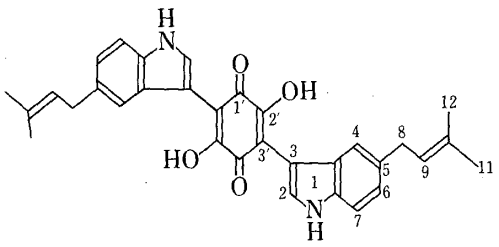
TABLE II. ^{1}H-NMR Data for Cochliodinol and Related Compounds (200 MHz, in THF-d_8)

	δ (ppm)										
	1-NH	2-H	4-H	5-H	6-H	7-H	8-H	9-H	11-H	12-H	2′-OH
Cochliodinol (**1**)	10.30 (br s) 2H	7.51 (d) 2H	7.39 (br s) 2H	—	6.90 (dd) 2H	7.19 (d) 2H	3.40 (d) 4H	5.36 (tqq) 2H	1.72 (br s) 12H		9.72 (s) 2H
Isocochliodinol (**2a**)	10.14 (br s) 2H	7.39 (d) 2H	7.38 (d) 2H	6.73 (dd) 2H	—	7.01 (d) 2H	3.32 (d) 4H	5.29 (tqq) 2H	1.639 (d) 6H	1.636 (d) 6H	9.56 (s) 2H
Neocochliodinol (**3a**)	10.28 (br s) 2H	7.52 (d) 2H	7.40 (dd) 2H	6.8—6.9 (m) 4H		—	[a]	5.48 (tqq) 2H	1.77 (d) 6H	1.72 (d) 6H	9.70 (s) 2H
					J (Hz)						
	1, 2	4, 5	4, 6	5, 6	5, 7	6, 7	8, 9	9, 11	9, 12		
Cochliodinol (**1**)	2.44	—	1.47	—	—	8.30	6.83	1.47	1.47		
Isocochliodinol (**2a**)	2.44	8.30	—	—	1.47	—	7.33	1.52	1.52		
Neocochliodinol (**3a**)	2.44	6.56	2.44	[b]	—	—	7.33	1.47	1.47		

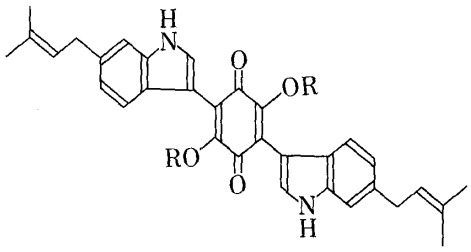
a) Overlapping with the solvent signal at δ 3.74.
b) Not observable.

indolyl)-benzoquinone (**2a**).

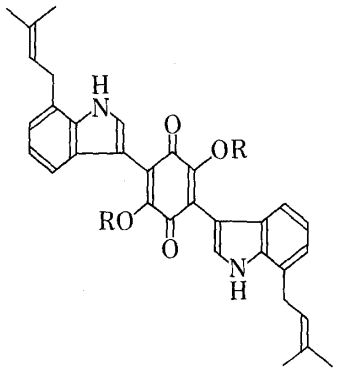
In the case of neocochliodinol (**3a**) the prenyl groups were expected to be attached to the 4- or 7-position of the rings. Although the chemical shift of the ring proton (δ 7.40), showing *ortho*- and *meta*-coupling, could be assigned to the proton at the 4-position, the results of the NOE study were obscure due to the overlapping of the signals of the other two aromatic protons. Thus, alkaline hydrogen peroxide oxidation of the hydrogenation product of neocochliodinol (**3a**) was performed. The product showing a positive spot with Ehrlich's reagent on thin-layer chromatography (TLC) was isolated and identified as 7-(3-methylbutyl)indole-3-carboxylic acid (**4**), obtained by the same procedures from asterriquinones



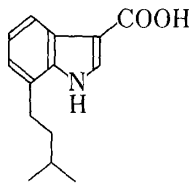
cochliodinol (**1**)



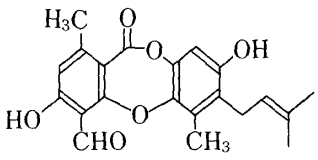
isocochliodinol (**2a**): R=H
isocochliodinol diacetate (**2b**): R=$COCH_3$



neocochliodinol (**3a**): R=H
neocochliodinol diacetate (**3b**): R=$COCH_3$
neocochliodinol dimethyl ether (**3c**): R=CH_3



7-(3-methylbutyl)-indole-3-carboxylic acid (**4**)



mollicellin G (**5**)

Chart 1

A-2, B-1, and C-2 from *Aspergillus terreus*.[3b] Thus, the structure of neocochliodinol was established as 2′,5′-dihydroxy-3′,6′-bis(7-(3-methyl-2-butenyl)-3-indolyl)-benzoquinone (**3a**).

Besides twelve kinds of asterriquinones from *A. terreus*,[3] a similar compound was recently isolated from *Nodulisporium hinnuleum*,[4] and 2′,5′-dihydroxy-3′,6′-bis(prenyl-3-indolyl)-benzoquinone derivatives isolated from molds now number sixteen.

The coexistence of cochliodinone,[2] isolated from *C. cochliodes* and *C. globosum* as a congener of cochliodinol, or of its positional isomers, was not detected by thin-layer chromatography under our conditions. A third species was also proved to produce mollicellin G (**5**), one of the mutagenic depsidones from *C. mollicellum*.[5] Although details of the chemical nature of the depsidones have not been published, comparison of the spectral data indicated identity of the present product with their compound.[5]

Experimental

Melting points were measured on a Yanagimoto micro-melting point apparatus and are uncorrected. UV, IR, and ^{1}H-NMR were measured with Hitachi 200-10, Nihon Bunko DS-403G, and JEOL FX-200 (199.5 MHz) machines, respectively. Chemical shifts are given in ppm from $(CH_3)_4Si$ added as an internal standard. MS were run on a JMS D300 FD mass spectrometer.

Isolation of Isocochliodinol (2a) from *C. murorum* (NHL (78-SH-271-4) and NHL 2240) and Neocochliodinol (3a) and Mollicellin G (5) from *C. amygdalisporum* (NHL 2874)——The procedures were reported in the previous paper.[1b]

Isocochliodinol (2a)——Recrystallized from tetrachloromethane–benzene, violet crystalline powder of mp 262 °C (dec.). UV, IR and NMR (Tables I and II). MS (FD) *m/z*: 506.0 (M^+, $C_{32}H_{30}N_2O_4$).

Isocochliodinol Diacetate (2b)——Isocochliodinol (30 mg) was treated with Ac_2O (1.0 ml) in pyridine (0.5 ml) at room temperature overnight. Recrystallization from benzene gave violet needles, mp 199—200 °C. IR (Table I). ^{1}H-

NMR δ (THF-d_8): 2.11 (6H, s, $COCH_3$), 1.79 (12H, br s, 11- and 12-CH_3), 3.42 (4H, d, J=6.5 Hz, 8-H), 5.42 (2H, m, 9-H), 6.97 (2H, dd, J=6.0, 2.5 Hz, 5-H), 7.21 (2H, br s, 7-H), 7.55 (2H, d, J=6.0 Hz, 4-H), 7.65 (2H, d, J=2.0 Hz, 2-H), 10.30 (2H, br s, 1-NH).

Neocochliodinol (3a)——Recrystallized from benzene–dichloromethane. Violet crystalline powder of mp 285 °C (dec.). UV, IR and NMR (Tables I and II). MS (FD) m/z: 506.0 (M^+, $C_{32}H_{30}N_2O_4$).

Neocochliodinol Diacetate (3b)——Neocochliodinol (20 mg) was treated with Ac_2O (1.0 ml) in pyridine (0.5 ml) at room temperature overnight. Recrystallization from benzene gave violet needles, mp 214 °C, UV and IR (Table I). ^{1}H-NMR δ (THF-d_8): 1.75 (12H, s, 11- and 12-CH_3), 2.06 (6H, s, $COCH_3$), 3.50 (4H, d, J=6.5 Hz, 8-H), 5.49 (2H, tqq, J=6.5, 1.45 Hz, 9-H), 6.9—7.0 (4H, m, 5- and 6-H), 7.46 (2H, dd, J=6.9, 2.3 Hz, 4-H), 7.62 (2H, d, J=2.3 Hz, 2-H), 10.50 (2H, br s, 1-NH).

Neocochliodinol Dimethyl Ether (3c)——Neocochliodinol (20 mg) was dissolved in ether (15 ml) and treated with ethereal diazomethane. After evaporation, the residue was purified by silica gel chromatography using chloroform as the developer. A violet crystalline powder of mp 237 °C was obtained. UV and IR (Table I). ^{1}H-NMR δ (THF-d_8): 1.72 (12H, br s, 11- and 12-CH_3), 3.74 (6H, br s, OCH_3), 3.57 (4H, d, J=6.0 Hz, 8-H), 5.48 (2H, tqq, J=6.5, 1.4, 1.4 Hz, 9-H), 6.5—7.0 (4H, m, 5- and 6-H), 7.35 (2H, dd, J=6.3, 2.9 Hz, 4-H), 7.56 (2H, d, J=2.9 Hz, 2-H), 10.50 (2H, br s, 1-NH).

Hydrogenation and Alkaline Hydrogen Peroxide Oxidation of Neocochliodinol (3a)——Neocochliodinol (60 mg) was dissolved in AcOEt (15 ml) and catalytically hydrogenated with 5% Pd-C (100 mg) at room temperature. After 4 h, three mol of H_2 had been absorbed. The catalyst was filtered off and the solvent was removed under reduced pressure. The residue was dissolved in 0.1 N NaOH (20 ml) and oxidized with 35% H_2O_2 (15 ml) at room temperature. After standing for 3 h, the solution was acidified and extracted with AcOEt. The extract was separated by preparative TLC (silica gel, hexane–AcOEt (1 : 1)). The compound at Rf 0.44 (positive to Ehrlich's reagent) was separated and crystallized from benzene as colorless needles (3 mg) of mp 195 °C, IR ν_{max}^{KBr} cm^{-1}: 3395, 2950, 1635 (br), 1528, 1440, 1315, 1171, 1150, 1120, 790, 750. Comparison with an authentic sample of 7-(3-methylbutyl)indole-3-carboxylic acid (**4**) confirmed its identity (IR spectra and TLC).

Mollicellin G (5)——Recrystallized from hexane–benzene, colorless needles, mp 198 °C, MS m/z: 368.1285 (M^+, Calcd for $C_{21}H_{20}O_6$, 368.1260). IR ν_{max}^{KBr} cm^{-1}: 3350, 1693, 1638, 1570, 1430, 1288, 1270, 1200, 1150, 1060, 850, 822, 767. ^{1}H-NMR δ (acetone-d_6): 1.65 (3H, s), 1.75 (3H, s), 2.33 (3H, s), 2.48 (3H, s), 3.55 (2H, d, J=6 Hz), 5.05 (1H, m), 6.67 (1H, s), 6.74 (1H, s), 8.73 (1H, s), 10.82 (1H, s), 12.26 (1H, s).

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References and Notes

1) *a*) S. Udagawa, T. Muroi, H. Kurata, S. Sekita, K. Yoshihira, S. Natori, and M. Umeda, *Can. J. Microbiol.*, **25**, 170 (1979); *b*) S. Sekita, K. Yoshihira, S. Natori, S. Udagawa, T. Muroi, Y. Sugiyama, H. Kurata, and M. Umeda, *ibid.*, **27**, 766 (1981); *c*) S. Sekita, K. Yoshihira, and S. Natori, *Chem. Pharm. Bull.*, **28**, 2428 (1980).
2) *a*) D. Brewer, W. A. Jerram, and A. Taylor, *Can. J. Microbiol.*, **14**, 861 (1968); D. Brewer, W. A. Jerram, D. Meiler, and A. Taylor, *ibid.*, **16**, 433 (1970); *b*) D. Meiler and A. Taylor, *ibid.*, **17**, 83 (1971); *c*) W. A. Jerram, A. G. McInnes, W. S. G. Maass, D. G. Smith, A. Taylor, and J. A. Walter, *Can. J. Chem.*, **53**, 727 (1975).
3) *a*) Y. Yamamoto, K. Nishimura, and N. Kiriyama, *Chem. Pharm. Bull.*, **24**, 1853 (1976); *b*) K. Arai, K. Masuda, N. Kiriyama, K. Nitta, Y. Yamamoto, and S. Shimizu, *ibid.*, **29**, 961 (1981).
4) M. A. O'Leary and J. R. Hanson, *Tetrahedron Lett.*, **23**, 1855 (1982).
5) A. A. Stark, B. Kobbe, K. Matsuo, G. Büchi, G. N. Wagan, and A. L. Demain, *Appl. Environ. Microbiol.*, **36**, 412 (1978); Professor G. Büchi, MIT, private communication.