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Syntheses and Cytotoxicity of Various 7-Methoxynogarene Derivatives

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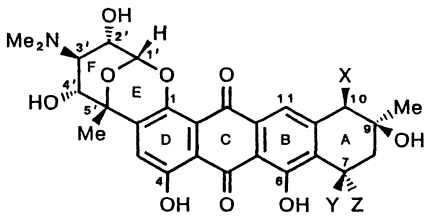
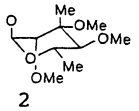
The title compounds were prepared employing the regioselective cycloaddition of the (+)-naphthoquinone derivative, the CDEF-ring system of nogalamycin, with various homophthalic anhydrides. Among the 7-methoxylnogarene derivatives obtained, 7-methoxy- and 9-hydroxy-7-methoxy-9-demethylnogarene and their 2',4'-diacetates were found to show prominent *in vitro* cytotoxicity against P388 murine leukemia.

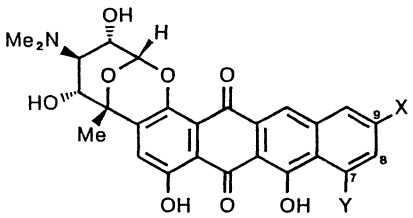
Nogalamycin (**1**) and its congeners are well-known as notable members of the anthracycline family due to their characteristic structures and prominent antitumor activity. Especially, (+)-7-con-*O*-methylnogarol (**2**), the semisynthetic derivative of **1**, has been subjected to clinical trials because of its broad spectrum activity and lower cardiotoxicity than that observed for adriamycin.[1)]

Previously, we reported the first total syntheses of the nogalamycin congeners such as (+)-nogarene (**4**), (+)-7-deoxynogarol (**3**), and (+)-7-con-*O*-methylnogarol (**2**), featuring the regioselective Diels-Alder reaction of the (+)-naphthoquinone (**5**), the CDEF-ring system of nogalamycin, with the dienes having the A-ring functionalities as a key step.[2)]

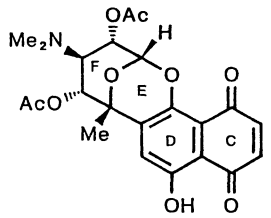
With completion of the total syntheses, the cytotoxicity assay was carried out with the nogalamycin congeners and their partial structures, leading to the conclusion that all the carbon framework (the ABCDEF-ring system) and the C-7 methoxy group are both indispensable for pronounced inhibitory activity.[3,4)] Furthermore, it appeared evident that, for the congeners carrying the C-7 methoxy group, absolute stereochemistry of the C-9 position also plays an important role for prominent cytotoxicity.[3)] In light of these results, it was of interest to



X = CO_2Me, Y = H, Z =  **1**
X = H, Y = OMe, Z = H **2**
X = Y = Z = H **3**



X = Me, Y = H **4**
X = H, Y = OMe **6**
X = Me, Y = OMe **7**
X = OBn, Y = OMe **8**
X = Y = OMe **9**
X = OH, Y = OMe **10**



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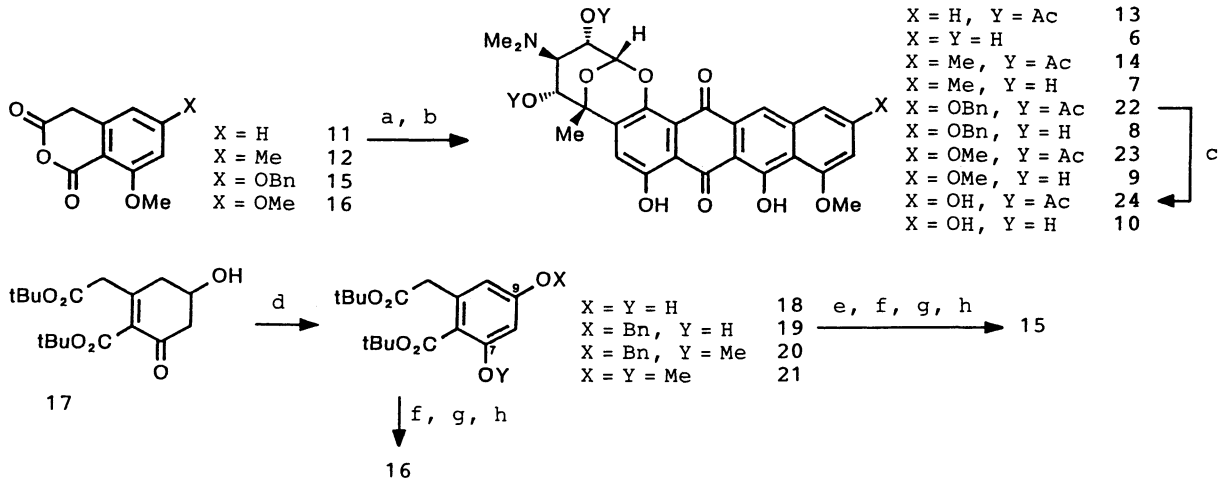
evaluate cytotoxicity of the 7-methoxynogarene derivatives (**6-10**) carrying various functionalities at the C-9 position. In this communication we wish to report the novel syntheses and cytotoxicity of **6-10** and their 2',4'-diacetates (**13, 14, 22-24**). Among these compounds, 7-methoxy- and 9-hydroxy-7-methoxy-9-demethylnogarene (**6** and **10**) and their diacetates (**13** and **24**) were found to show potent *in vitro* cytotoxicity against P388 murine leukemia.

For the systheses of **6-10**, it was anticipated that their 11-deoxy-anthracyclinone skeletons could be effectively constructed from **5** by employing the strong base induced cycloaddition of homophthalic anhydrides explored by Tamura *et al.*[5] Although it had been established during the course of our total syntheses of **2-4**[2b,c,6] that the Diels-Alder reactions of **5** under the neutral conditions cleanly occurred in a completely regioselective manner, the base induced cycloaddition of homophthalic anhydrides had not been examined with **5**.

Thus, the base induced cycloadditions of the well-known homophthalic anhydrides (**11** and **12**)[7] with **5** were first attempted. Similarly to the reported results,[5,7] the reactions of **5** with the sodium salts of **11** and **12** were found to take place smoothly in a completely regioselective manner, affording the diacetates (**13** and **14**) after air oxidation of the addition products during workup, **13**: mp 175-178 °C; $[\alpha]_D^{20}$ +533° (c 0.030, $CHCl_3$), and **14**: mp 250-255 °C (decomp.); $[\alpha]_D^{20}$ +545° (c 0.110, $CHCl_3$). Both diacetate (**13** and **14**) were deprotected, giving 7-methoxy-9-demethylnogarene (**6**), mp 230-235 °C, $[\alpha]_D^{20}$ +400° (c 0.030, $CHCl_3$), and 7-methoxynogarene (**7**), mp 231-235 °C (decomp.), $[\alpha]_D^{20}$ -900° (c 0.070, $CHCl_3$), respectively.

Next, syntheses of **8-10** carrying the oxygen functionalities at the C-9 position were examined. Syntheses of the requisite homophthalic anhydrides (**15**



a) 1) NaH, THF, rt, 20 min 2) **5**, 0 °C→rt, 1 h, 62% (**13**, from **5**), 81% (**14**, from **5**), 100% (**22**, from **5**), 96% (**23**, from **5**) b) K_2CO_3, MeOH, 40 °C, 30 min (for **13** and **14**), MeOH-$CHCl_3$, rt, 5 h (for **22-24**), 78% (**6**), 72% (**7**), 63% (**8**), 96% (**9**), 18% (**10**) c) H_2, Pd-$BaSO_4$, MeOH, rt, 2 h, 79% d) Jones reagent, Me_2CO, 0 °C, 2 h, 89% e) BnBr, $K_2CO_3 \cdot 1.5H_2O$, Me_2CO, reflux, 2 h, 83% f) Me_2SO_4, K_2CO_3, Me_2CO, reflux, 3 h, 95% (**20**), 98% (**21**) g) CF_3CO_2H, CH_2Cl_2, rt, 12 h h) Ac_2O, PhMe, 100 °C, 20 min, 99% (**15**, 2 steps), 98% (**16**, 2 steps).

and **16**) were performed using the keto diester (**17**)[8] as the starting material. Thus, the keto diester (**17**) was first converted to the diphenol (**18**), mp 124-126 °C, by Jones oxidation. After selective benzylation[9] of the C-9 hydroxyl group of **18**, the remaining C-7 hydroxyl group of the benzyl ether (**19**) was methylated to yield the diether (**20**). Cleavage of the two *tert*-butyl esters followed by dehydration of the resulting dicarboxylic acid, afforded **15**, mp 171-172 °C. On the other hand, after methylation of the two hydroxyl groups of **18**, similar sequential treatments converted the dimethyl ether (**21**) into **16**, mp 167-168 °C.

The regioselective cycloadditions of **5** with the sodium salts of **15** and **16** followed by air oxidation of the addition products during workup similarly gave rise to the diacetates (**22** and **23**), **22**: mp 251-254 °C; $[\alpha]_D^{20}$ +706° (c 0.051, $CHCl_3$), and **23**: mp 174-177 °C; $[\alpha]_D^{20}$ +649° (c 0.111, $CHCl_3$). Deacetylation of **22** and **23** readily produced 9-benzyloxy-7-methoxy-9-demethylnogarene (**8**), mp >270 °C (decomp.), $[\alpha]_D^{20}$ -1780° (c 0.009, $CHCl_3$), and 7,9-dimethoxy-9-demethylnogarene (**9**), mp 228-231 °C, $[\alpha]_D^{20}$ -1040° (c 0.025, $CHCl_3$), respectively. Removal of the benzyl group of **22** by hydrogenation gave the diacetate (**24**), mp >290 °C, $[\alpha]_D^{20}$ -2500° (c 0.020, $CHCl_3$), which on further deprotection afforded 9-hydroxy-7-methoxy-9-demethylnogarene (**10**).[10]

These 7-methoxynogarenes (**6-10**) and their 2',4'-diacetates (**13**, **14**, **22-24**) were subjected to *in vitro* cytotoxicity assay against P388 murine leukemia. Results are shown in Table 1. It appeared that, among the tested samples, **6**, **10**, and their 2',4'-diacetates (**13** and **24**) exhibited prominent cytotoxicity compared well with that of **3** and its diacetate [IC_{50} = 0.003-0.012 μg/ml (for **3**) and 0.014 μg/ml (for the diacetate of **3**)]. The 2',4'-diacetates (**13** and **14**) being chemically more stable than the corresponding diols (**6** and **10**) have been subjected to *in vivo* test for antitumor activity against P388 murine leukemia. These results will be reported shortly.

Table 1. *In Vitro* Cytotoxicity of 7-Methoxynogarenes (**6-10**) and Their 2',4'-Diacetates (**13**, **14**, **22-24**)

7-Methoxynogarenes		7-Methoxynogarene 2',4'-Diacetates	
Compound	IC_{50} (μg/ml)[a]	Compound	IC_{50} (μg/ml)[a]
6	0.040	**13**	0.038
7	0.057	**14**	0.13
8	0.13	**22**	0.18
9	0.043	**23**	0.11
10	0.032	**24**	0.016

a) Cell growth inhibition (percent) after incubation for 48 h at 37 °C.

We are grateful to Dr. K. Sakai, Sagami Chemical Research Center, Drs. S. Tsukagoshi and T. Tashiro, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, for evaluation of *in vitro* cytotoxicity and *in vivo* antitumor activity against P388 murine leukemia cells.

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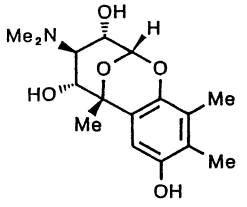
1) P.F. Wiley, "*Anthracycline Antibiotics*," ed by H.S. El Khadem, Acedemic

Press, New York (1982), p. 97.

2) a) M. Kawasaki, F. Matsuda, and S. Terashima, *Tetrahedron Lett.*, 26, 2693 (1985); b) M. Kawasaki, F. Matsuda, and S. Terashima, *ibid.*, 27, 2145 (1986); c) M. Kawasaki, F. Matsuda, and S. Terashima, *ibid.*, in press.

3) The nogalamycin congeners (**2-4**) were subjected to *in vitro* cytotoxicity assay against P388 murine leukemia along with their related compounds such as 7,9-di-*epi*-7-con-*O*-methylnogarol (**i**), 9-*epi*-7-deoxynogarol (**ii**), and 7,8-dihydronogarene (**iii**), which had been obtained in the course of our total syntheses of **2-4**.[2b,c] Following IC_{50} (μg/ml) values were recorded: **2**, 0.003-0.012; **3**, 0.41; **4**, 0.11; **i**, 0.40; **ii**, 0.31; **iii**, 0.13. The 7-demethoxy congeners (**3**, **4**, **ii**, and **iii**) were found to exhibit marginal *in vitro* cytotoxicity. No significant *in vivo* antitumor activity against P388 murine leukemia was observed for **ii** and **iii**. It had been reported that **3** and **4** exhibited no activity in P388 *in vivo* test.[1] In contrast to **2** and its C-7 epimer, 7-dis-*O*-methylnogarol, showing potent antitumor activity against P388 murine leukemia *in vitro* and *in vivo*,[1] the marginal cytotoxicity was only observed for the 9-*epi*-congener (**i**).

4) *In vitro* cytotoxicity assay against P388 murine leukemia was carried out on the DEF-ring system (**iv**),[2a] CDEF-ring system (**5**),[2b] and BCDEF-ring systems (**v** and **vi**).[6] The partial structures (**iv-vi**) except for **5** showed no significant cytotoxicity [IC_{50} (μg/ml): **iv**, >10; **v**, 1.5; **vi**, 1.6]. Although marginal cytotoxicity was observed on **5** (IC_{50} = 0.10 μg/ml), it showed no inhibitory activity against P388 murine leukemia *in vivo*.



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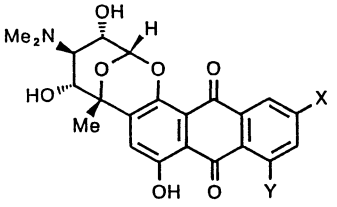
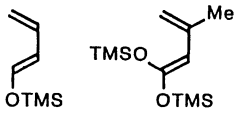


X = Y = H v
X = Me, Y = OH vi

5) Y. Tamura, M. Sasho, S. Akai, A. Wada, and Y. Kita, *Tetrahedron*, 40, 4539 (1984) and references cited therein.

6) The Diels-Alder reactions of **5** with the linear dienes (**vii** and **viii**) were found to proceed in a completely regioselective manner. Removal of the two acetyl groups of the addition products readily produced the BCDEF-ring systems (**v** and **vi**), **v**: 80% (from **5**); mp 254-264 °C (decomp.); $[\alpha]_D^{20}$ +707° (c 0.230, $CHCl_3$), and **vi**: 52% (from **5**); mp 259-261 °C (decomp.); $[\alpha]_D^{20}$ +820° (c 0.120, $CHCl_3$).



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7) Y. Tamura, F. Fukata, T. Tsugoshi, M. Sasho, Y. Nakajima, and Y. Kita, *Chem. Pharm. Bull.*, 32, 3259 (1984).

8) M. Yamaguchi, *Yuki Gosei Kagaku Kyokai Shi*, 45, 969 (1987).

9) R.N. Hurd and D.H. Shah, *J. Org. Chem.*, 38, 607 (1973).

10) The low yield of **10** is probably due to lability of **10** under the conditions for deacetylation. Since **10** was sufficiently pure (*ca.* 90% by ^1H NMR), it was directly subjected to *in vitro* cytotoxicity assay.

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