IMPROVED ANTITUMOR ACTIVITY BY MODIFICATION OF NOGALAMYCIN[1]

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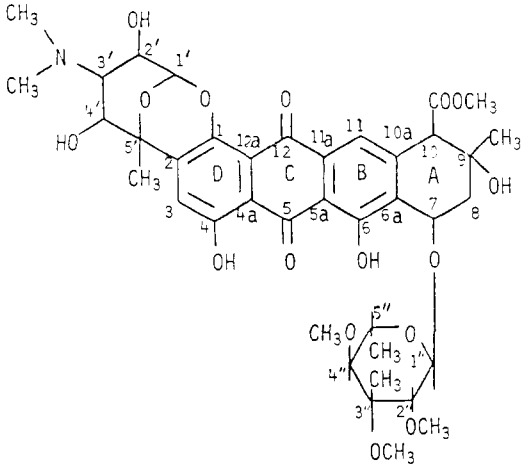
ABSTRACT.—Nogalamycin, an antitumor antibiotic, has been converted to a series of analogs by removal of the carbomethoxy group at C–10 and replacement of the neutral sugar at C–7 by other groups. Removal of the carbomethoxy group to give disnogamycin (**6**) followed by acidic alcoholysis gave pairs of isomeric 7-alkoxy compounds differing in configuration at C–7. Treatment of **6** with trifluoroacetic acid followed by nucleophiles gave a series of analogs having substituents at C–7 with a configuration at C–7 opposite to that of nogalamycin. Among the analogs prepared, 7-*con*-*O*-methylnogarol (**7**) is a highly active antitumor agent.

The antibiotic nogalamycin, whose gross structure is that indicated as **1**, was isolated about 15 years ago by R. B. Kelly at The Upjohn Company (1). It was a highly active gram-positive antibacterial agent also having considerable activity against KB cells and L1210 cells *in vitro*. It was found to be active against two solid tumors *in vivo*, but its relatively low activity accompanied by difficulties in administration caused cessation of testing.



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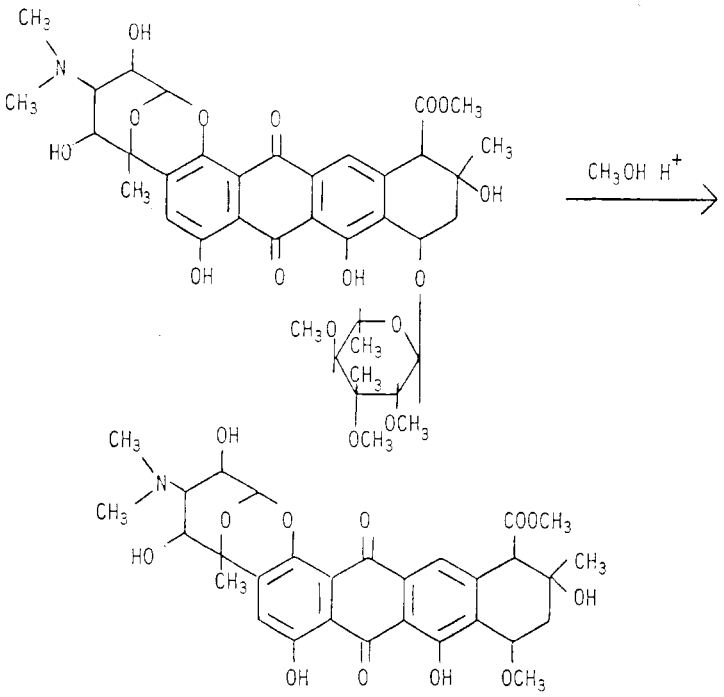
During the period of interest in nogalamycin, structural studies were continued and degradation products obtained were tested. One of these, 7-dis-*O*-methylnogalarol, was found to have properties which indicated superiority to nogalamycin as an antitumor agent. Unfortunately, its superiority was not striking enough to bring about extensive investigation. Its structure and mode of preparation are indicated in scheme 1. In the original work only one isomer was isolated, but subsequently a second one was found to be present.

At this point it is desirable to clarify the stereochemistry of nogalamycin and

[1]Presented as a plenary lecture at the 20th Annual Meeting of the American Society of Pharmacognosy at Purdue University, West Lafayette, Indiana, July 30–August 3, 1979.

Scheme 1



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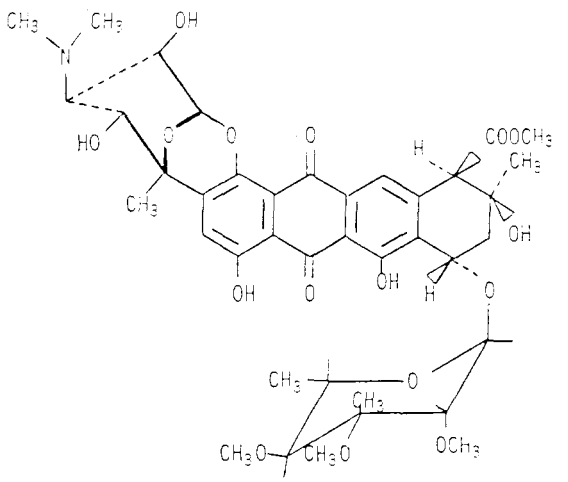
7-DIS-O-METHYLNOGALAROL: OH at C-9 trans to CH_3O at C-7

7-CON-O-METHYLNOGALAROL: OH at C-9 cis to CH_3O at C-7

its analogs. The structure of nogalamycin was originally published (2) with considerable uncertainty as to stereochemistry. The complete stereochemistry of the neutral sugar, nogalose, was known; and it was known that the aminosugar moiety had the configuration of either α-L- or α-D-glucopyranose. It was suggested on the basis of reactions and pmr spectra that the configuration in ring A was either 7S, 9S, 10R or 7R, 9R, 10S. The latter seemed more probable as the 9R configuration has thus far been universal in anthracyclines. Subsequently, on the basis of circular dichroism (cd) curves following the work of Brockmann *et al.* (3), the configuration 7S, 9R, 10R was assigned and analogs were named on this basis. For example, the compound now called 7-dis-*O*-methylnogalarol was thought to have the 7(S) configuration and was named accordingly. Recently, Stezowski (4) has determined the gross structure and relative stereochemistry of 7-con-*O*-methylnogarol (7) by X-ray crystallography. It was shown that the oxygen atoms at C–7 and C–9 are on the same side of ring A and that the aminosugar moiety is on the opposite side of the linear tetracyclic system. Since this analog has a configuration at C–7 opposite to that in nogalamycin, the sugar moiety at C–7 must be on the side of ring A away from the C–9 hydroxyl group. Thus, the two sugars must be on the same side of the linear tetracyclic ring system. Cd studies strongly suggest that if C–7 is S in nogalamycin, the carbomethoxy group

at C–10 is on the side of ring A opposite to nogalose (5). These studies also indicate that C–7 is S in nogalamycin (5), as is usually the case in anthracyclines. All of these findings would be consistent with the structure indicated as **3** in which C–7 is S, C–9 is S, and C–10 is R with the amino sugar having the α-L configuration. At present it is believed that this is the structure of nogalamycin. However, the fact in all cases of anthracyclines previously studied for configuration at C–9, the configuration is R and that the α-D-glucopyranose configuration would be somewhat more likely makes it seem possible that the mirror image of **3**, i.e. **4**,



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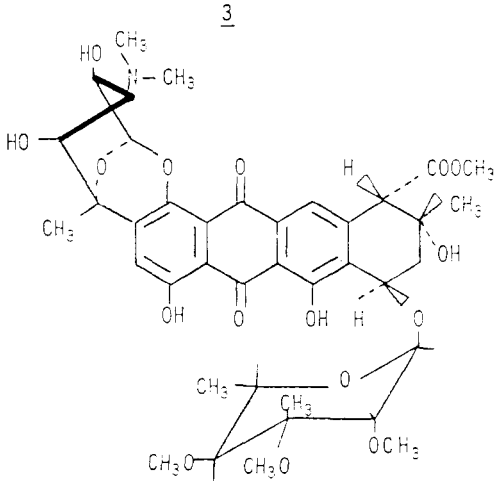

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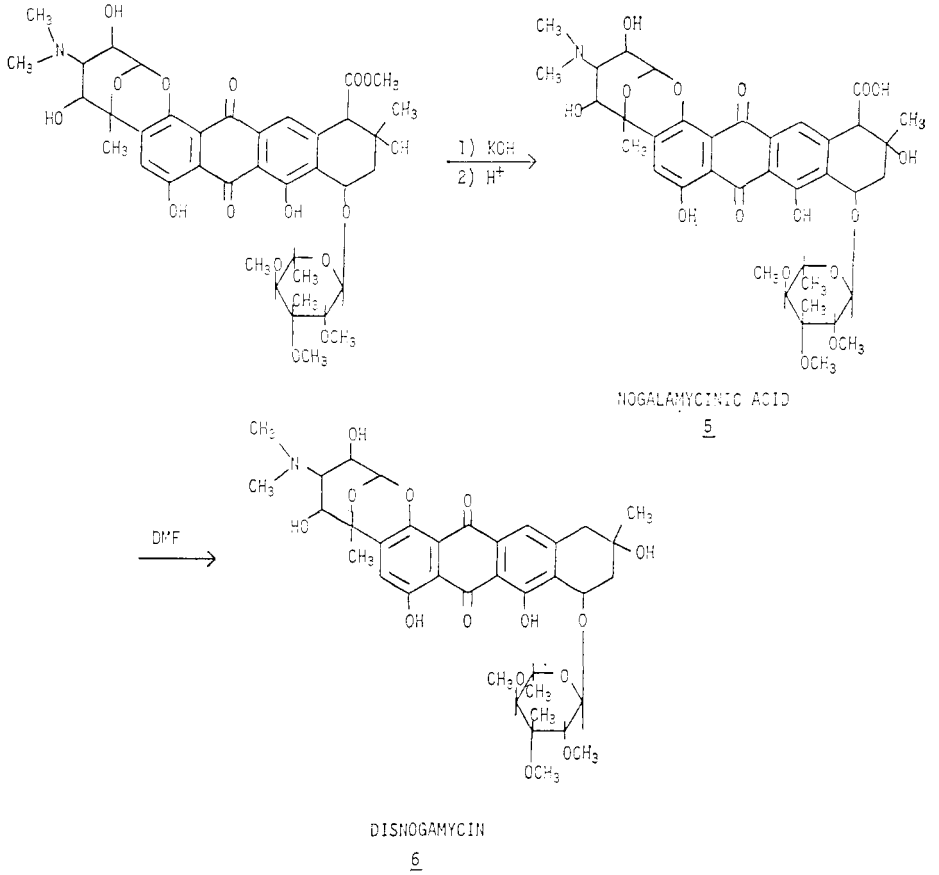
might be the correct structure. Therefore, it seems best to wait for complete crystallographic data to report the total absolute stereochemistry with certainty. At present it can be said that any structure other than **3** and **4** is highly improbable. The series of analogs having oxygen atoms *cis* at C–7 and C–9 are now distinguished by the prefix con while the series having these oxygen atoms *trans* are referred to as dis.

Some years after the original biological studies on nogalamycin, a revival of interest in this antibiotic occurred, and a large number of analogs were prepared. It was found that very mild basic hydrolysis formed a compound in which the carbomethoxy group at C–10 had been hydrolyzed as indicated in scheme 2. The unionized acid is indicated, but **5** exists as the zwitterion. The acid loses carbon dioxide extremely readily by solution in DMF to give disnogamycin (**6**). The

Scheme 2



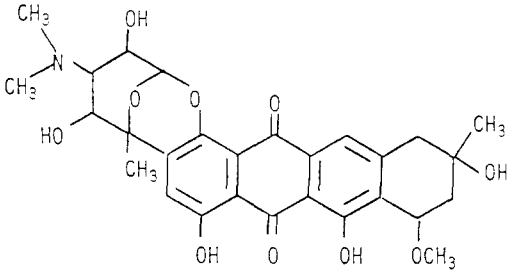
NOGALAMYCINIC ACID

5

DISNOGAMYCIN

6

latter compound was substantially more active than nogalamycin in our *in vivo* P388 leukemia screen. Since 7-dis-*O*-methylnogalarol (**2**) had been very interesting, the analogous conversion was carried out with disnogamycin. The result was the isolation of two compounds (**7** and **8**) in which the neutral sugar was replaced by methoxyl. These products were obtained by a short period of heating of a solution of disnogamycin in methanol about 0.5 *N* in hydrogen chloride. The products were isomeric at C–7 with one having the S configuration and the other the R; however, as previously mentioned, definite assignments of absolute configuration cannot be made at this time. Available experimental evidence is certainly in favor of the R configuration for the compound called 7-con-*O*-methylnogarol (**7**) in which case C–9 would also be R. Configuration at C–7 in **7** was shown by



7, 7-con-O-methylnogarol

8, 7-dis-O-methylnogarol

circular dichroism studies (5) to have been inverted relative to the C–7 configuration in disnogamycin. The ratio of compounds **7** and **8** formed in the reaction was about 6:4. Purification proved to be quite difficult as aromatization of ring A occurred quite readily, solubility was a problem, and removal of solvents was difficult. The structure of the aromatization product, nogarene, is indicated in **9**. As a consequence of these difficulties, yields of pure 7-con-*O*-methylnogarol, which proved to be the desired isomer, were only in the neighborhood of 25% for the overall sequence from nogalamycin.

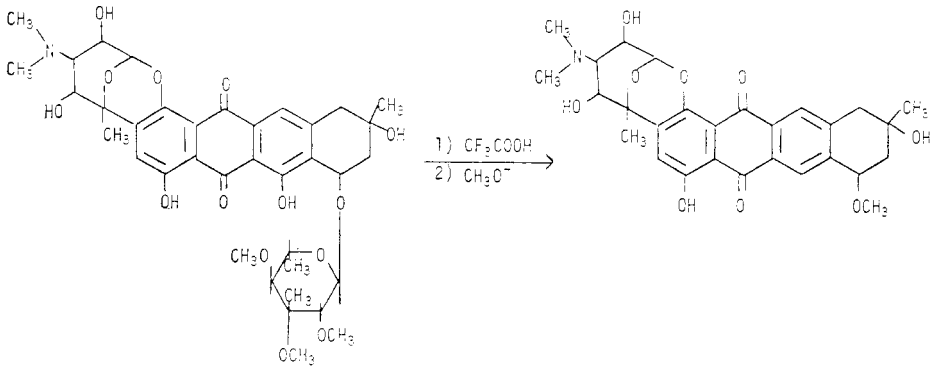
It was found that similar pairs of isomeric 7-*O*-alkylnogarols and 7-*O*-alkylnogalrols could be readily prepared using ethanol, *n*-propanol, and *iso*-propanol. Purification of the longer chain analogs was easier than that of the *O*-methyl compounds because of improved solubility in organic solvents and greater ease of separation from ring A aromatized compounds.

The two compounds in each pair exhibit characteristic differences. The dis isomers (*trans* oxygen at C–7 and C–9) are less mobile in silica gel tlc plates using the solvent system chloroform-methanol-water (78:20:2) than are the con isomers. In the nogarol series in the ^{13}C nmr spectra of each pair, the chemical shift for C–6a in the dis isomer is about $\delta 1.5$ downfield from that of the con isomer (*cis* oxygen at C–7 and C–9), while the signal for C–10 is about $\delta 1$ upfield. The isomeric pairs in the nogarols show similar differences.

Brockmann and Niemeyer (6) have reported the isomerization of various anthracyclinones at C–7 by reaction with trifluoroacetic acid followed by hydrolysis. A somewhat similar reaction of disnogamycin (6) was found to occur. Solution of **6** in trifluoroacetic acid at temperatures of ~15° to 25° gave a product which, although never completely characterized, reacted with nucleophiles to introduce the nucleophile at C–7 with essentially complete stereospecificity (at least 98%) to the compound having the opposite chirality at C–7. In the case in which the nucleophile is methoxide, the reaction is shown in scheme 3. As far as is known at present, the reaction is general for nucleophiles. It occurs with alkoxides, including nogalose anion, acid anions, mercaptides, ammonia, primary and secondary amines, azides, and carbanions. It was found that 7-con-*O*-methylnogarol reacts with trifluoroacetic acid to give a product which reacts with ethoxide ion to form 7-con-*O*-ethylnogarol. This suggests that the overall reaction always results in a con product regardless of chirality at C–7 in the initial reactant.

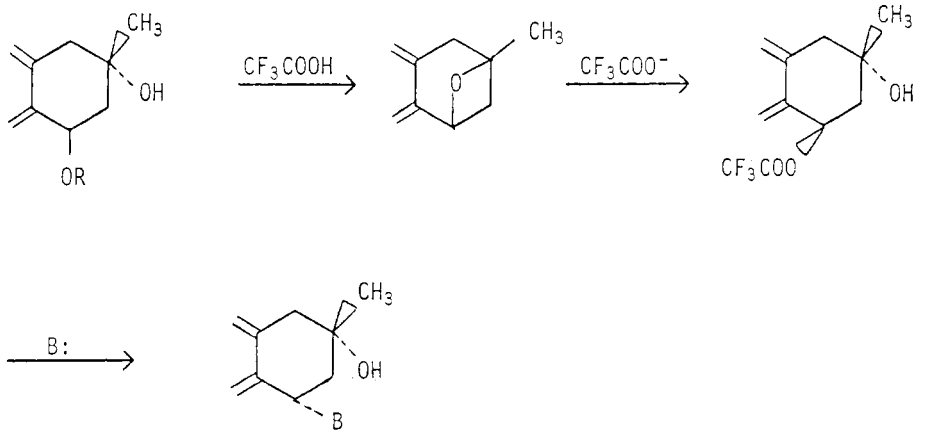
The nature of the product obtained in the first step is somewhat uncertain, as

Scheme 3



purification difficulties prevented its characterization. However, it was found that the intermediate contains a trifluoroacetate moiety bound in some fashion other than as a salt. It seems probable that the intermediate is a trifluoroacetyl ester of disnogarol which then undergoes an S_N 2 reaction with nucleophiles to form compounds of the con series (scheme 4). In order to result in the stereospecificity found, the chirality at C–7 of the intermediate must be influenced by the hydroxyl group at C–9. Possibly an intermediate oxetane ring is formed, or its equivalent consisting of an ion pair, which can then react with CF_3COO^-. The indicated stereochemistry in scheme 4 is intended to be only relative and not absolute, and only ring A is depicted.

Scheme 4



The trifluoroacetic acid procedure provides an excellent method for preparing a large number of nogalamycin analogs, but only members of the con series can be prepared in this fashion. However, it is not generally applicable to anthracyclines or their analogs other than those of the nogamycin type. The same procedure applied to nogalamycin and steffimycin gave only very poor results. A

mixture of products was formed with the corresponding anthracyclinone being predominant.

About 50 analogs of nogalamycin have been prepared, and virtually all of them showed considerable activity as antitumor agents. 7-Con-*O*-methylnogarol (**7**) was the most active of the compounds prepared, and discussion will focus on it and its close analogs. However, there will first be given some very limited results from compounds having substituents other than alkoxy at C–7 (table 1). All of these were prepared by the trifluoroacetic acid procedure and are in the con series. The compounds were tested against P388 leukemia in mice on a schedule in which the tumor was inoculated on Day 0, and the dosage indicated was given daily for 9 days. The activities of these compounds are not outstanding, although they are in the range of nogalamycin. The most interesting point in this group is the low toxicity of the amino compounds. The limiting factor in administering nogalamycin analogs *in vivo* is their toxicity, and dosages reported are usually the highest that can be given without severe toxicity. In the connogamycin series the 7-amino compounds are strikingly less toxic than other types tested, as can be seen by the large size of doses given compared to those given for acetoxy, methylmercapto, and azido analogs (table 1).

TABLE 1.[a] *In vivo* activity of acetoxy, mercapto, and amino analogs.

Substituent at C–7[b]	Dose (mg/kg)[c]	% ILS
CH_3COO	10	87
CH_3S	8	37
CH_3NH	25	58
$(CH_3)_2N$	50	54
C_2H_5NH	25	55
$(C_2H_5)_2N$	25	38
N_3	5	33

[a]P388 leukemia cells were inoculated into mice IP on Day 0, and drug was injected IP daily for 9 days thereafter.
[b]All compounds were in the con series.
[c]The dosage given is that resulting in the highest ILS.

As mentioned previously, the *in vivo* activity of disnogamycin was somewhat greater than that of nogalamycin, and this fact was the original impetus towards preparing and investigating its analogs. Table 2 gives *in vitro* and *in vivo* data

TABLE 2. *In vitro* and *in vivo* activities of compounds containing nogalose.[a]

Compound	L1210 *in vitro*		P388 Leukemia		L1210 Leukemia	
	ID_{50}[b]	ID_{95}[b]	Dose[c]	ILS	Dose[c]	ILS
Nogalamycin	0.078	0.18	1	49	1	30
Nogalamycinic acid	1.00	1.94	20	56	20	18
Disnogamycin	0.18	0.27	5	93	2.5	27

[a]*In vivo* results were obtained by Day 0 IP inoculation of tumor with IP injection of drugs daily for the subsequent 9 days.
[b]Expressed in mg/ml.
[c]mg/kg/day.

for nogalamycinic acid and disnogamycin and compares their activities with that of nogalamycin (7). Data are given for *in vitro* and *in vivo* activity against L1210 which is another murine leukemia and P388. The differences in activity against L1210 *in vivo* were not striking, but against P388 disnogamycin was a highly active compound. It is interesting to note that *in vitro* activities correlate very poorly with *in vivo* activities.

A series of six 7-*O*-alkylnogarols, three of each isomer, was prepared along with a series of nogalarols; both series were prepared by acidic alcoholysis. As indicated in table 3, activities *in vitro* against L1210 cells do not vary greatly since

TABLE 3. *In vitro* activities of 7-alkyoxynogalarols and nogarols.[a]

C-7 Substituent	Nogalarol Series		Nogarol Series	
	Dis ID_{50}	Con ID_{50}	Dis ID_{50}	Con ID_{50}
CH_3O	0.27	0.13	0.41	0.061
C_2H_5O	0.16	0.15	0.43	0.16
n-C_3H_7O	0.16	0.30	0.33	0.10

[a]Activities against L1210 cells expressed as the concentration in μMoles necessary to inhibit cell growth by 50%.

they are all within one order of magnitude, although in general the con isomers in the nogarol series seem to be somewhat more active than the dis isomers. As in most other systems, 7-con-*O*-methylnogarol is the most active compound of the group.

Among this group of compounds the *O*-methylnogalarols and *O*-methylnogarols were the first compounds tested *in vivo*. As a result of these tests done against P388 leukemia in mice, it was decided that the nogarols were more active than the nogalarols and the con isomers were more active than the dis. Consequently, not all of the above alkoxy compounds were tested *in vivo*. The activities of those tested against P388 murine leukemia are shown in table 4 as well as the results for 7-con-*O*-*iso*propylnogarol prepared by the trifluoroacetic acid procedure. The marked superiority of 7-con-*O*-methylnogarol is apparent in these results.

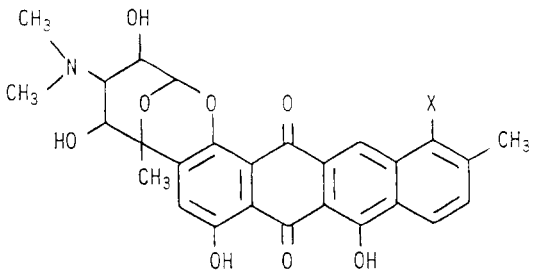
TABLE 4. Activities of 7-alkoxynogalarols and nogarols against murine P388 leukemia.[a]

Substituent	Nogalarol Series				Nogarol Series			
	Dis		Con		Dis		Con	
	Dose[b]	% ILS	Dose[b]	% ILS	Dose[b]	% ILS	Dose[b]	% ILS
CH_3O	25	60	25	98	12.5	76	12.5	197
C_2H_5O	—	—	12.5	70	—	—	6.5	63
n-C_3H_7O	—	—	—	—	3.1	50	6.3	34
iso-C_3H_7O	—	—	—	—	—	—	4	20

[a]P388 leukemia cells injected IP on Day 0 and drug injected IP daily on Days 1–9.
[b]mg/kg/day.

Not included in the table is the fact that it effected three cures while none of the other compounds gave any cures. Although the data are rather limited, it is clear that activity is inversely proportional to the size of the 7-*O*-alkyl group and that con isomers are more active than dis isomers.

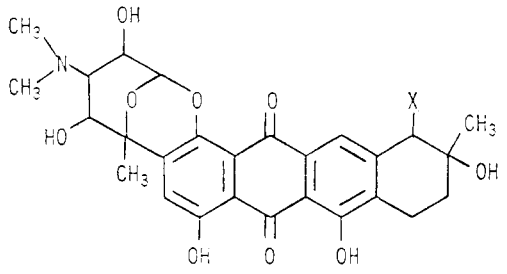
Four compounds have been prepared in which oxygen at C–7 is totally lacking. In two of these (**9** and **10**) complete aromatization of ring A has occurred, thus



9, Nogarene, X = H

10, Nogalarene, X = $COOCH_3$

eliminating both substitutents containing oxygen. These were prepared by acid treatment of nogalamycin and disnogamycin. These are nogarene (**9**) and nogalarene (**10**). The other compounds of this group are 7-deoxynogalarol (**11**) and 7-deoxynogarol (**12**) in which the oxygen-containing substituent at C–7 has



11, 7-DEOXYNOGALAROL, X = $COOCH_3$

12, 7-DEOXYNOGAROL, X = H

been replaced by hydrogen. Catalytic reduction of nogalamycin or of any of the nogalarols gives 7-deoxynogalarol. This in turn was converted to 7-deoxynogarol by the hydrolysis and decarboxylation procedure used in obtaining disnogamycin (scheme 2). The L1210 *in vitro* and P388 activity *in vivo* of these compounds is shown in table 5. *In vitro* activity was found to be rather similar to that of other compounds already mentioned, but *in vivo* activities had essentially disappeared except for nogalarene.

The results obtained in L1210 *in vitro* testing and in P388 murine leukemia *in vivo* testing focused our attention on 7-con-*O*-methylnogarol. Most of the other compounds were not tested against L1210 leukemia *in vivo* since this is not done

TABLE 5. Activities of 7-deoxy and ring A aromatized compound.

Compound	L1210 *in vitro*[a]		P388 Leukemia[b]	
	ID_{50}	ID_{90}	Dose	% ILS
Nogalarene	0.24	0.58	70	50
Nogarene	0.22	0.37	12.5	17
7-Deoxynogalarol	0.58	1.51	50	27
7-Deoxynogarol	0.94	1.76	100	21

[a]Activities expressed as the concentration in μM necessary to kill 50% of the cells.
[b]Done as reported previously. Dose is mg/kg/day.

routinely in our laboratories as are the P388 tests. Table 6 reports L1210 *in vivo* activity for 7-con-*O*-methylnogarol and one compound in the nogalarol series. As can be seen from the table, 7-con-*O*-methylnogarol is highly active; and, in addition, one cure was seen.

TABLE 6. Activities against L1210 leukemia *in vivo*.[a]

Compound	Dose (mg/kg/day)	% ILS
7-Dis-*O*-methylnogalarol	50	12
7-Con-*O*-methylnogarol	12.5	140

[a]L1210 cells injected IP on Day 0 and drug injected IP daily on Days 1-9.

The next step in testing antitumor agents in our laboratories is testing against the B16 melanoma in mice. The results found in the B16 melanoma tests are shown in table 7. In these tests a number of compounds were found to be quite active. Both nogalamycin and disnogamycin had good activity as did both dis and con 7-*O*-methylnogarols and 7-dis-*O*-methylnogalarol. However, the con isomer of the latter compound had little activity. The 7-*O*-ethylnogarols were also much less active than were the methyl analogs.

TABLE 7. Activities against B16 melanoma *in vivo*.[a]

Compound	B16 Melanoma	
	Dose (mg/kg/day)	% ILS
Nogalamycin	0.5–1	71–157
Disnogamycin	2–5	61–260
7-Dis-*O*-methylnogalarol	50	84
7-Con-*O*-methylnogalarol	10	35
7-Dis-*O*-methylnogarol	16	105
7-Con-*O*-methylnogarol	6–12	77–109
7-Dis-*O*-ethylnogarol	12	34
7-Con-*O*-ethylnogarol	12	25

[a]Tumors were given IP on Day 0 and drug was injected IP on Days 1-9. Ranges of activity are given when several experiments were done.

At this point 7-con-*O*-methylnogarol looked like a very active compound; and, in order to find out how good it was, it was compared directly with adriamycin in a series of tests. This was done because adriamycin is the best antitumor antibiotic known at the present time. The results are shown in table 8. Most of

TABLE 8.[a] Comparative antitumor activity of 7(R)-*O*-methylnogarol and adriamycin.

Tumor		7-Con-*O*-methylnogarol		Adriamycin	
System	Site	Dose[b]	% ILS[c]	Dose[b]	% ILS[c]
P388	IP	25	T	2	7
		12.5	168 (1)	1	158 (1)
		6.25	111	0.5	137 (1)
		3.13	61	0.25	61
P388	IV	25	T	2	29
		12.5	187	1	10
		6.25	53	0.5	3
		3.13	25	0.25	8
L1210	IP	25	T	2	40 (2)
		12.5	104 (1)	1	38 (1)
		6.25	55	0.5	29
		3.13	42	0.25	20
B16	IP	20	−9	2	158
		10	114	1	166
		5	88	0.5	160
		2.5	64	0.25	127

[a]Taken from Neil *et al.*, Reference 7.
[b]mg/kg/injection, Q1Dx9 (1), IP.
[c]Increase in life span calculated from median survival times; numbers in parenthesis are cures.

these tests were done in the same fashion as were those already discussed; i.e., intraperitoneal injection of both tumor cells and drug. In both of the leukemias, 7-con-*O*-methylnogarol compared quite favorably with adriamycin in activity, although the potency was lower by a factor of about ten. Although 7-con-*O*-methylnogarol was quite active in the B16 melanoma test, it was somewhat less active than was adriamycin. In one experiment the procedure was altered with P388 cells being administered iv while the drug was injected ip. When both tumor cells and drug are given ip, it is possible that activity is a result of direct contact or of very high local concentration. This possibility was eliminated by the combination of iv-ip injection. In this experiment 7-con-*O*-methylnogarol was substantially better than adriamycin. In addition, it has been found that 7-con-*O*-methylnogarol has oral activity giving a % ILS of 48 at 12.5 mg/kg while adriamycin lacks such activity.

Since the results already discussed suggested considerable promise, 7-con-*O*-methylnogarol was studied further at the National Cancer Institute in a battery of seven tumors, three being repeats of those already done at The Upjohn Company. The results shown in table 9 are a portion of the data reported from the National Cancer Institute. The activities for P388 and L1210 leukemia and B16 melanoma are quite comparable to those already mentioned, with good activity being seen in all three systems. Activity was also quite good against two colon tumors and a mammary tumor. Only marginal activity was found against Lewis lung carcinoma. The criterion of activity against this system is 40%ILS.

TABLE 9. Mouse tumor-therapy studies with 7-con-*O*-methylnogarol.[a]

Tumor		Schedule[c]	Dose (mg/ko-inj)	% ILS (cures)
System	Site[b]			
P388	IP	Q1Dx9 (1)	12.5	197 (2)
L1210	IP	Q1Nx9 (1)	12.5	140 (1)
Colon 26	IP	Q4Dx2 (1)	25	106 (4)
Lewis Lung	IP	Q1Dx9 (1)	12.5	38
B16	IP	Q1Dx9 (1)	12.5	109 (T/C cures)[d]
Colon 38	SC	Q7Dx2 (2)	25	21 (3)
CD8F1	SC	Q7Dx5 (1)	25	1
Mammary				

[a]Tests done at the National Cancer Institute.
[b]Site of tumor inoculatian.
[c]Drug injected IP Q1Dx9 (1) means drug was given daily for 7 days starting one day after tumor infection. Q4Dx2 (1) means drug was given every fourth day for two injections starting one day after tumor inoculation,
[d]Ratio of median tumor size of treated and control groups + 100.

A very serious problem arising in the clinical use of adriamycin is its cumulative cardiotoxicity. The largest amount of adriamycin which can be given to a patient is about 500 mg/m^2 of body surface without danger of irreversible, fatal cardiotoxicity. As a consequence of this property of adriamycin, the cardiotoxicity of 7-con-*O*-methylnogarol was investigated by the standard procedure using rabbits as the test animal (8). In order to make these cardiotoxicity tests comparable with those of adriamycin, 7-con-*O*-methylnogarol was administered at approximately 15 times the dose at which adriamycin causes death (table 10). At this level substantially less cardiotoxicity was seen than was seen with adriamycin. The severity of pathology in each animal was rated on a scale of 0 to 4 and the average was taken. The value found for adriamycin was 3.3 while that for 7-con-*O*-methylnogarol was 2.5 (table 10).

TABLE 10, Cardiotoxicity of 7-con-*O*-methylnogarol compared with that of adriamycin.

Compound	Cumulative Dose (mg-m^2)	Score
Adriamycin	222-277	3.3
7-Con-*O*-methylnogarol	3700	2.5

In order to study the biological properties of the nogalamycin analogs further, their effect on macromolecular synthesis and DNA binding was investigated in L1210 leukemia cells (9). Nogalamycin strongly inhibits RNA synthesis, and to a somewhat lesser degree, DNA synthesis; however, it does not inhibit protein synthesis. The results with 7-con-*O*-methylnogarol (table 11) against DNA and RNA synthesis in L1210 cells were quite surprising. It almost totally failed to inhibit either RNA or DNA synthesis. The dis isomer was much less inhibitory than nogalamycin but did inhibit both RNA and DNA synthesis. In the cases of the other two pairs of con and dis isomers, the con was much less inhibitory than

TABLE 11, Inhibitian of macromolecular synthesis by nogalamycin and its analogs.

Compound	ID_{50} (nmole/ml)[a]	
	NA	RNA
Nogalamycin	3.3	0.4
Disnogamycin	3	0.5
7-Dis-*O*-methylnogalarol	3.8	0.5
7-Con-*O*-methylnogalarol	7–17	7.8
7-Dis-*O*-methylnogarol	11.6	8.3
7-Con-*O*-methylnogarol	>46	>46
7-Dis-*O*-ethylnogarol	3.2	0.8
7-Can-*O*-ethylnogarol	10.6	5.2

[a]Concentration causing 50% inhibition.

was the dis. No inhibition of protein synthesis was seen with any of the compounds listed in table 11.

Similar studies with L1210 ascites cells were done (table 12) with a few of the compounds shown in Table 11. Once again the great difference in inhibition of RNA synthesis was found, as can be seen from table 12. A concentration of most of these compounds of 10 nmol/ml resulted in virtually complete repression of RNA synthesis, while 7-con-*O*-methylnogarol at this concentration exhibited no effect on RNA synthesis.

TABLE 12. Inhibition of RNA synthesis in L1210 ascites cells.

Compound	Drug concentration (nmole/ml)	% Inhibition of RNA synthesis
Nogalamycin	10	99.9
Nogalamycinic acid	10	86.7
7-Dis-*O*-methylnogarol	10	91.2
7-Con-*O*-methylnogarol	10	0

The interaction of calf thymus DNA with nogalamycin and its analogs was measured by circular dichroism (cd). The difference between cd curves at 465 nm of DNA and of the compound taken separately and the cd curve of the mixture was determined and expressed as millidegrees of ellipticity (table 13). The values

TABLE 13. Nogalamycin and its analogs interaction with DNA.

Compound	Drug-DNA interaction[a] (millidegrees of ellipticity)
Nogalamycin	12
Disnogamycin	11
7-Dis-*O*-methylnogalarol	8
7-Con-*O*-methylnogalarol	6
7-Dis-*O*-methylnogarol	8
7-Con-*O*-methylnogarol	2
7-Dis-*O*-ethylnogarol	8
7-Con-*O*-ethylnogarol	2

[a]Difference spectrum determined by circular dichroism at 465 nm.

shown are proportional to drug-DNA interaction. It is immediately apparent that the two compounds in the connogarol series bind much less strongly to DNA than do compounds in the dis series. The effect is seen to some extent in the nogalarol series (7-con-*O*-methylnogalarol vs 7-dis-*O*-methylnogalarol), but it is much less pronounced.

The familar DNA melting point increase technique for determining DNA binding also indicates failure of the 7-con-*O*-methylnogarol to bind to DNA. Disnogamycin raises the melting point of DNA by 11.7° while the con-*O*-methyl analog has no effect.

In summary, it can be said that substantial modification of nogalamycin at C–10 and C–7 has given a number of compounds having antitumor activity in the range of the parent compound. It has also led to a compound, 7-con-*O*-methylnogarol, which has outstanding antitumor properties and whose biochemical properties differ greatly from those of the other anthracyclines. 7-Con-*O*-methylnogarol is not only highly active but has substantially reduced cardiotoxicity and seems likely to be clinically effective.

ACKNOWLEDGMENTS

I wish to thank Mrs. Jian Johnson, Mr. David J. Houser, and Mr. David W. Elrod for most of the chemical work discussed. I also wish to express by appreciation for the use of biological data developed by Dr. L. H. Li, Dr. J. Patrick McGovren, and Dr. Gary L. Neil and their associates. My thanks are extended to Dr. William C. Krueger who did the circular dichroism experiments. A portion of the biological results were furnished by the National Cancer Institute. This work was supported in part by Contract N01-CM-77110 and previous contracts from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Education, and Welfare.

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