NOGALAMYCIN ANALOGS HAVING IMPROVED ANTITUMOR ACTIVITY

Sir:

Nogalamycin (**1**)[1,2] is an anthracycline antibiotic which has activity against Gram-positive microorganisms and is an antitumor agent.[3] However, because of its rather modest activity and undesirable side effects, it never became a clinically useful agent. It appeared likely that modification of **1** would give compounds superior in some respects to the parent compound since this has already been achieved by conversion of **1** to 7-O-methylepinogalarol (**2**)[2,3]. As a consequence, a program of modification was initiated which has led to agents which have been shown to be superior to **1** and at least the equal of adriamycin, the present standard in antitumor chemotherapy, in antitumor activity.[4,5] The present communication describes the conversion of **1** to nogalamycinic acid (**3**), nogamycin (**4**), and 7-O-methylnogarol (**5**), and preliminary data on their biological activity.

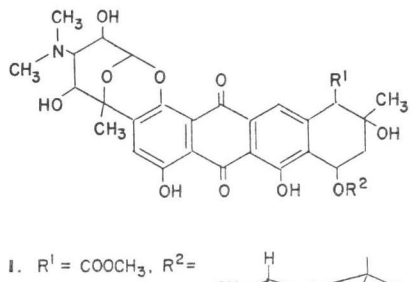
Solution of **1** in 0.53 N KOH solution for 16 hours followed by acidification (H_2SO_4) gave the free acid (**3**). The crude product contained considerable inorganic material, but it was suitable for conversion to **4**. Purification of **3** by column chromatography on silica gel using gradient elution with $CHCl_3$ - CH_3OH gave a red solid, mp 219~229°C; Rf ($CHCl_3$ - CH_3OH - H_2O; 78: 20: 2) 0.25; $[\alpha]_D$ +456° (*c* 0.37, CH_3OH); UV (EtOH) λ_{max} 236 nm (ε 39,950), 269 (ε 21,350), 291 sh (ε 8,700), 482 (13,550); IR (Nujol) 3450, 1670, 1630, 1595, 1580, 1290, 1230, 1215, 1135, 1095, 1060, 1015, 980, 920, 855, 830, 780, 763, and 725 cm^{-1}; mass spectrum *m/e* 729 (M $-CO_2$); H^1 NMR ($CDCl_3$-CD_3OD) δ1.38 (m, 9H, $3CH_3C$), 1.80 (s, 3H, CH_3C), 3.15 [s, 6H, $(CH_3)_2NH^+$], 3.38, 3.40, 3.68 (3s, 9H, $3CH_3O$), 3.2~4.0 (m, CHO, and CHN), 5.24, 5.88 (2d, 2H, anomeric), 6.92, 7.47 (2s, 2H, aromatic); ^{13}C NMR ($CDCl_3$-CD_3OD) δ16.4, 19.3, 24.6, 31.5 ($4\underline{C}H_3C$), 42.5 [$(CH_3)_2N$], 49.9, 58.2, 60.3 (3 CH_3O), 62.5~85.9 (10C, CHO, CHN), 97.3, 100.2 (anomeric), 113.1~161.4, (12C, aromatic), 178.9, 181.6, 191.4 (carbonyl). The analysis and mass spectra of **3** did not establish its molecular formula, but conversion to **2** by treatment at room temperature with methanolic hydrogen chloride (0.4 N) and determination of the molecular formula of **4** combined with physical data defined the structure as **3** except that it exists as a zwitterion rather than as the free acid indicated.

When **3** was dissolved in DMF at room temperature for 16 hours, CO_2 was given off (identified as $BaCO_3$) and **4** was formed. Evaporation of the solvent gave a crude residue purified by chromatography on silica gel using gradient elution with $CHCl_3$ - CH_3OH (yield of **1** to **3** about 25%). Recrystallization from CH_3COCH_3 - CH_3OH (85: 15) gave a red solid, mp 210~215°C; Rf (solvent as above) 0.50; $[\alpha]_D$ +273° (*c* 0.923, $CHCl_3$); UV (EtOH) λ_{max} nm 236 (ε 51,700), 259 (ε 25,850), 290 (ε 10,050), 478 (ε 16,100); IR (Nujol) 3500, 1670, 1630, 1575, 1295, 1230, 1110, 1055, 1005, 920, 890, 838, 778, 702, and 724 cm^{-1}; mass spectrum *m/e* 729; ^1H NMR (d_7-DMF) δ1.14, 1.23, 1.37, 1.69 (12H, $4CH_3C$), 2.07~2.38, 2.83~3.0 (m, 4H, $2CH_2$), 2.42 [s, 6H, $(CH_3)_2N$], 3.13, 3.42, 3.52 (3s, 9H, $3CH_3O$), 3.3~4.2 (m, CHO, CHN), 4.95 (m, 1H, benzylic CHO), 5.32, 5.68 (2d, 2H, anomeric), 7.16, 7.32 (2s, 2H, aromatic); ^{13}C NMR ($CDCl_3$) δ15.2, 18.3, 24.2, 30.4 (4 $\underline{C}H_3C$), 30.8, 44.1 ($2CH_2$), 41.5 [$(CH_3)_2N$], 48.7, 59.0, 61.4 ($3CH_3O$), 66.4~88.6 (10C, CHO, CHN), 96.9, 99.8 (anomeric), 113.1~161.4 (12C, aromatic), 179.7, 190.8 (carbonyl).

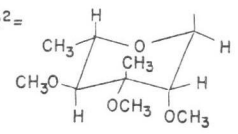
Anal. Calcd. for $C_{37}H_{47}NO_{14}$: C, 60.96; H, 6.55; N, 1.92. Found: C, 58.55; H, 6.42; N, 1.94.

Methanolysis of **4** with boiling methanolic hydrogen chloride (0.4 N) for about 2 hours gave **5**. Purification by chromatography on silica gel

Fig. 1



1. R^1 = $COOCH_3$, R^2=



2. R^1 = $COOCH_3$, R^2 = CH_3

3. R^1 = COOH, R^2 = R^2 in **1**

4. R^1 = H, R^2 = R^2 in **1**

5. R^1 = H, R^2 = CH_3

($CHCl_3$ - CH_3OH; 95: 5) gave a 53% yield of a red solid; mp 248~253°C; Rf (same solvent as above) 0.64; $[\alpha]_D$ +958° (*c* 0.163, $CHCl_3$); UV (EtOH) λ_{max} nm 235 (ε 41,200), 251 (ε 25,500), 257 (ε 24,150), 290 (ε 10,500), 479 (ε 15,530); IR (Nujol) 3470, 1675, 1625, 1580, 1470, 1430, 1405, 1385, 1300, 1230, 1135, 1115, 1085, 1065, 1015, 950, 925, 890, 870, 850, and 790 cm^{-1}; mass spectrum *m/e* 541; ^1H NMR ($CDCl_3$-CD_3OD) δ 1.45, 1.73 (2s, 6H, CH_3C), 2.32~2.50, 2.73~3.1 (m, 4H, $2CH_2$), 2.58 [s, 6H, $(CH_3)_2N$], 3.60 (s, 3H, CH_3O), 3.3~4.2 (m, CHO, CHN), 4.83 (m, 1H, benzylic H), 5.82 (d, 1H, anomeric), 6.77, 7.25, (2s, 2H, aromatic); ^{13}C NMR ($CDCl_3$-CD_3OD) δ23.9, 30.0 (2 CH_3C), 36.1, 44.1 ($2CH_2$), 41.6 [$(CH_3)_2N$], 57.9 (CH_3O), 66.1~75.2 (6C, CHO, CHN), 97.6 (anomeric), 112.6~161.1 (12C, aromatic), 179.7, 190.9 (carbonyl).

Anal. Calcd. for $C_{28}H_{31}NO_{10}$: C, 62.10; H, 5.78; N, 2.59. Found: C, 62.21; H, 5.94; N, 2.66.

Much more extensive biological data has been reported,[4,5] but as an indication of activity, Table 1 gives results obtained against P388 leukemia in mice.

Table 1. Activity of nogalamycin analogs against mouse leukemia (P388)*

Compound	Dose (mg/kg/day)	% ILS
1	2.0	40
3	10	38
4	10	67
5	50	155
Adriamycin	1.0	57

* All agents were administered ip days, 1, 5, and 9 after ip leukemia inoculation on day 0 except for adriamycin which was given on days 1~9. Inoculum was 10^6 P388 cells/mouse given on day 0. Median survivals were 10.2~10.7 days in the different experiments represented.

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References

1) BHUYAN, B. K. & A. DIETZ: Fermentation, taxonomic, and biological studies of nogalamycin. Antimicr. Agents & Chemoth. -1965: 836~844, 1966
2) WILEY, P. F.; R. B. KELLY, E. L. CARON, V. H. WILEY, J. H. JOHNSON, F. A. MACKELLAR & S. A. MIZSAK: Structure of nogalamycin. J. Amer. Chem. Soc. 99: 542~549, 1977
3) BHUYAN, B. K. & F. REUSSER: Comparative biological activity of nogalamycin and its analogs. Cancer Res. 30: 984~989, 1970
4) NEIL, G. L.; C. L. BLOWERS, D. J. HOUSER, J. H. JOHNSON & P. F. WILEY: Antitumor activity of some new analogs of nogalamycin. American Association for Cancer Research, Annual Meeting, Denver, Colorado, May 18~21, 1977
5) LI, L. H.; B. K. BHUYAN & W. C. KRUEGER: Comparative biological and biochemical effects of nogalamycin and its analogs on L1210 mouse leukemia. American Association for Cancer Research, Annual Meeting, Denver, Colorado, May 18~21, 1977