68.5, 67.5, 66.8, 58.9, 48.3, 43.0, 40.2, 39.2, 32.8, 32.5, 25.9, 25.6, 23.8, 18.1, -4.4, -4.7; $[\alpha]_D$ -20.1° (c 0.318, $CHCl_3$) (lit.[11m,33] $[\alpha]_D$ -27.73° (c 1.44, $CHCl_3$)).

Methyl (1*R*,2*R*,4*S*)-4-[(2-Methoxyethoxy)methoxy]-2-((*S*)-6-hydroxy-1-heptenyl)-α-[(*S*)-(2-methoxyethoxy)methoxy]cyclopentane-β-butenoate (26). Following the procedure of Corey and Carpino,[11m,33] 3:1 acetic acid/water (0.8 mL) was added to silyl ether **25** (9.47 mg, 1.57 × 10^{-5} mol). After 5.5 h, the solution was partitioned between ether and saturated aqueous Na_2CO_3. The organic layers were dried ($MgSO_4$), concentrated, and chromatographed to give **26** as a colorless oil (5.97 mg, 78%): R_f (50% EtOAc/petroleum ether) = 0.10, R_f (EtOAc) = 0.40; ^1H NMR ($CDCl_3$) δ 6.81 (dd, J = 6.15, 15.76 Hz, 1 H), 5.94 (dd, J = 1.0, 15.8 Hz, 1 H), 5.36 (m, 2 H), 4.68 (s, 2 H), 4.67 (s, 2 H), 4.21-4.12 (m, 2 H), 3.81-3.59 (m, 1 H), 3.72 (s, 3 H), 3.67-3.64 (m, 4 H), 3.55-3.50 (m, 4 H), 3.38 (s, 3 H), 3.37 (s, 3 H), 2.34 (m, 1 H), 2.17 (m, 1 H), 2.0-1.3 (m, 11 H), 1.17 (d, J = 6.17 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 166.6, 148.2, 133.6, 130.9, 121.3, 94.3, 94.2, 76.7, 75.9, 71.8, 71.7, 67.9, 67.5, 66.9, 59.0, 51.6, 48.3, 43.1, 40.3, 38.8, 33.2, 32.4, 25.6, 23.5.

Bis(MEM) Brefeldin A (27). Continuing the procedure of Corey and Carpino,[11m,33] 1 M aqueous LiOH (73.3 μL) was added to a solution of ester **26** (5.97 mg, 1.11 × 10^{-5} mol) in methanol (0.43 mL). After 21 h, the mixture was partitioned between chloroform and 10% aqueous HCl. The organic layers were dried ($MgSO_4$), concentrated, and chromatographed to give the hydroxy acid as a pale green oil (crude wt 5.47 mg): R_f (EtOAc) = 0.03; ^1H NMR ($CDCl_3$ δ 6.86 (dd, J = 6.2, 15.76 Hz, 1 H), 5.94 (d, J = 16.0 Hz, 1 H), 5.32 (m, 2 H), 4.69 (s, 2 H), 4.67 (m, 2 H), 4.15 (m, 2 H), 3.78 (m, 2 H), 3.66 (m, 4 H), 3.55 (m, 5 H), 3.38 (s, 3 H), 3.37 (s, 3 H), 2.31 (m, 1 H), 2.16 (m, 1 H), 2.0-1.2 m, 10 H), 1.18 (d, J = 6.0 Hz, 3 H).

Triphenylphosphine (9.4 mg, 3.58 × 10^{-5} mol) was added to a solution of the hydroxy acid (crude wt 5.47 mg), xylenes (0.224 mL, 0.05 M), and "Aldrithiol 2" (Aldrich) (2,2'-dipyridyl disulfide) (8.22 mg, 3.73 × 10^{-5} mol). After 7 h, the solution was diluted with xylenes (5.47 mL) and then warmed to reflux for 12 h. The solution was washed with 10% aqueous HCl, dried ($MgSO_4$), concentrated, and chromatographed to give an oil (2.54 mg), which still contained pyridyl residues. This oil was partitioned between ether and 10% aqueous HCl. The organic layers were dried ($MgSO_4$), concentrated, and chromatographed to yield **27** as an oil (1.15 mg, 21% from methyl ester **26**): R_f (EtOAc) = 0.48; ^1H NMR ($CDCl_3$) δ 7.08 (dd, J = abc, 1 H), 5.83 (d, J = abs, 1 H), 5.62 (m, 1 H), 5.21 (m, 1 H), 4.95-4.6 (m, 4 H), 4.2-4.05 (m, 2 H), 3.83 (m, 1 H), 3.66 (m, 4 H), 3.54 (m, 4 H), 3.38 (s, 6 H), 2.35-1.5 (m, 12 H), 1.24 (d, J = 6.13 Hz, 3 H).

Preparation of 27 from Natural (+)-Brefeldin A. MEM chloride (6.5 μL) was added to a solution of natural (+)-brefeldin A (Sandoz) (approximately 4 mg), CH_2Cl_2 (1.25 mL), and *N*,*N*-diisopropylamine (9.9 μL). After 45 h, the mixture was partitioned between CH_2Cl_2 and brine. The organic layers were dried (Na_2SO_4), concentrated, and chromatographed. The bis(MEM) product **27** eluted first, as a yellow oil (1.33 mg). Next eluted was the mono(MEM) product as an oil (0.61 mg). Mono(MEM): R_f(mono(MEM)) (25% acetone/ether) = 0.54; ^1H NMR ($CDCl_3$) δ 7.34 (dd, J = 3.0, 15.65 Hz, 1 H), 5.90 (dd, J = 2.0, 15.69 Hz, 1 H), 5.69 (m, 1 H), 5.245 (m, 1 H), 4.9-4.6 (m, 2 H), 4.2-4.05 (m, 2 H), 3.75-3.63 (m, 2 H), 3.57 (m, 3 H), 3.38 (d, J = 1.23 Hz, 3 H), 2.35-2.1 (m, 4 H), 2.1-1.95 (m, 2 H), 1.91-1.66 (m, 6 H), 1.25 (d, J = 6.21 Hz, 3 H). Bis(MEM): R_f(bis(MEM)) (25% acetone/ether) = 0.64, R_f (EtOAc) = 0.48; UV/vis λ 235 nm; ^1H NMR ($CDCl_3$) δ 7.09 (dd, J = 3.67, 15.75 Hz, 1 H), 5.83 (d, J = 15.63 Hz, 1 H), 5.62 (m, 1 H), 5.20 (m, 1 H), 4.95-4.6 (m, 4 H), 4.2-4.05 (m, 2 H), 3.83 (m, 1 H), 3.65 (m, 4 H), 3.54 (m, 4 H), 3.38 (s, 6 H), 2.35-1.5 (m, 12 H), 1.24 (d, J = 6.28 Hz, 3 H).

(+)-Brefeldin A (3). Concluding the procedure of Corey and Carpino,[11m,33] a 1 M solution of titanium tetrachloride in CH_2Cl_2 (0.115 mL) was added dropwise to a solution of synthetic **27** (1.15 mg, 2.52 × 10^{-6}) in CH_2Cl_2 (0.115 mL) at 0 °C. After 1.17 h at 0 °C, the reaction was quenched with saturated aqueous $NaHCO_3$. The solution was partitioned between chloroform and brine. The organic layers were dried ($MgSO_4$), concentrated, and cospotted on TLC with natural (+)-brefeldin A. The spots matched exactly. The concentrate was then chromatographed to give **3** as a white solid: R_f (25% acetone/ether) = 0.375; ^1H NMR ($CDCl_3$) (partial) δ 7.35 (dd, J = 2.99, 15.36 Hz, 1 H), 5.90 (dd, J = 1.71, 15.79 Hz, 1 H).

Acknowledgment. We thank Sandoz Pharma Ltd., Basel, for a sample of natural (+)-brefeldin A (**3**) and Professor E. J. Corey for sharing with us his detailed experimental procedures for the conversion of **19** to **3**. This work was supported by the Center for Catalytic Science and Technology of the University of Delaware.

Isolation and Characterization of the Duocarmycin-Adenine DNA Adduct

Dale L. Boger,* Takayoshi Ishizaki,[1] and Hamideh Zarrinmayeh

Contribution from the Department of Chemistry, Purdue University, West Lafayette, Indiana 47907. *Received December 27, 1990*

Abstract: The isolation and full characterization of the duocarmycin-adenine adduct **7** derived from the duocarmycin alkylation of calf thymus DNA is detailed.

Two independent efforts have described the isolation, structure determination, assignment of absolute configuration, and preliminary evaluation of a new class of antitumor antibiotics now including duocarmycin A[2-4] (**1**), duocarmycin B_1 and B_2 (**2** and **3**),[6] duocarmycin C_1[3-5] (**4**, pyrindamycin B[7]), duocarmycin C_2[3] (**5**, pyrindamycin A[7]), and duocarmycin SA (**6**).[8] Figure 1. The structural similarity between duocarmycin A (**1**) and (+)-CC-1065 suggested that the agents may be acting by a common or related

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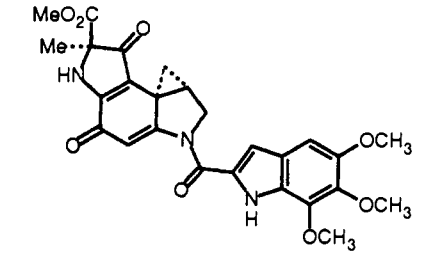
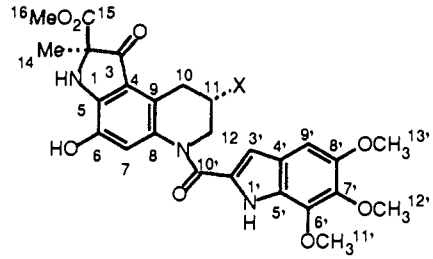
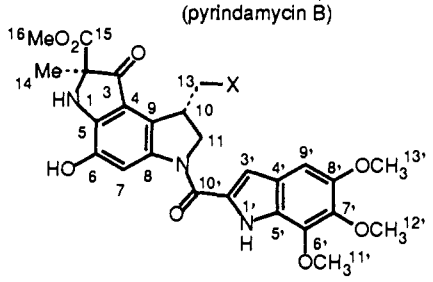
0002-7863/91/1513-6645$02.50/0



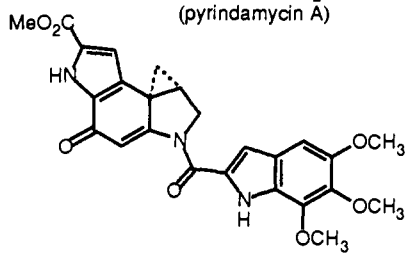
1 duocarmycin A



2 duocarmycin B_1 X = Br
4 duocarmycin C_1 X = Cl
(pyrindamycin B)



3 duocarmycin B_2 X = Br
5 duocarmycin C_2 X = Cl
(pyrindamycin A)



6 duocarmycin SA

Figure 1.

mechanism initiated with the irreversible alkylation of DNA. In recent efforts, we have disclosed studies detailing the event and selectivity of the duocarmycin alkylation of DNA that provided support for the potential that the agents may be acting by such a common mechanism.[9,10] In these studies the thermally induced strand cleavage of double-stranded DNA after exposure to the agents[11-15] was employed to demonstrate the DNA adenine N-3 alkylation at concentrations comparable to that of (+)-CC-1065 and was used to define the duocarmycin DNA alkylation sequence selectivity.[10,11] Herein, we report the isolation and full characterization of the duocarmycin–adenine covalent adduct **7**[10] derived from the thermal depurination reaction of double-stranded DNA which serves to unambiguously establish the nature of the predominant alkylation of double-stranded DNA, Scheme I.

Table I. Calf Thymus DNA Alkylation

agent	alkylation conditions[a]	% 7[b]	% recovered agent[c]
1[d]	24 °C, 7 days, 54 bp equiv DNA	90	7
4[d]	24 °C, 7 days, 50 bp equiv DNA	21	69
4[d]	24 °C, 7 days, 150 bp equiv DNA	40	47
5[d]	4 °C, 48 h, 50 bp equiv DNA	20	79
5[d]	24 °C, 7 days, 50 bp equiv DNA	33	61
5[d]	24 °C, 7 days, 290 bp equiv DNA	65	32
5[e]	24 °C, 7 days, 140–150 bp equiv DNA	73	21

[a] Calf thymus DNA (shredded, sonicated, and soluble), 0.1 M Na_2HPO_4/NaH_2PO_4 (pH 7.4), 25 °C. [b] The amount of **7** recovered from 1-butanol extracts of thermally treated (100 °C, 25 min) solutions (1:1 0.1 M aqueous Na_2HPO_4/NaH_2PO_4–*n*-BuOH). [c] The amount of unreacted starting agent recovered from the alkylation reaction (EtOH) following precipitation of the DNA. [d] Analytical scale, UV determination of **7** and recovered **1**, **4**, and **5**. [e] Preparative scale, isolation of **7** and recovered **5**.

Optimized conditions for near complete alkylation of calf thymus DNA[16] (type I, Sigma) were established for duocarmycin C_2 on an analytical scale (10–200 μg agent), and representative results are summarized in Table I. In these studies, a survey comparison of duocarmycin A and C_1–C_2 established that the three agents provide the same adenine adduct **7** thermally released from DNA following alkylation (HPLC and TLC comigration: R_T = 22 min, 10 × 250 mm SiO_2, 16% MeOH–CH_2Cl_2, 2.5 mL/min; R_f (SiO_2) = 0.2, 10% MeOH–$CHCl_3$; identical UV). For this purpose, the long wavelength UV absorption of the agents and adduct outside the UV absorption range of DNA provided a useful quantitative measure of the extent of alkylation (DNA bound **7** or isolated **7**) and the amount of recovered, unbound agent. As established in prior studies,[10] the relative rate of adenine N-3 alkylation for the three agents with double-stranded DNA follows the order of duocarmycin A > C_2 > C_1, and the observation of the same adduct from A and C_1 requires C_1 to close to A prior to or concurrent with alkylation. Employing the conditions estimated to provide near complete alkylation of DNA for duocarmycin C_2 (**5**), the preparative isolation of the adduct **7** was accomplished. Thus, precipitation of the calf thymus DNA (EtOH) following alkylation by duocarmycin C_2 (3.18 mg, 24 °C, 7 days, 140 bp equiv DNA) afforded 0.66 mg (21%) of recovered **5** from the ethanol supernatant. Thermal treatment of the alkylated calf thymus DNA in aqueous buffer (100 °C, 25 min; 2×) followed by *n*-butanol extraction and subsequent purification of the thermally released adduct by chromatography (Sephadex LH-20) provided **7** (2.74 mg, 73%, 92% based on recovered **5**). Thus, the adenine N-3 alkylation of duocarmycin C_2 quantitatively accounts for 92% (86–92%) of the consumption of the agent in the presence of double-stranded DNA and constitutes the predominant and near exclusive alkylation event.

The full physical and spectroscopic characterization of the adduct led to the unambiguous assignment of the structure **7** in which adenine N-3 addition to the unsubstituted cyclopropane carbon of duocarmycin A was established. Most notably, the ^1H NMR (Table II), 2D ^1H–^1H COSY NMR, 2D ^1H–^1H NOESY NMR (Figures 2 and 3), and ^{13}C NMR (Table III) spectra of **7** along with the comparative spectroscopic properties of **2**–**5** and **8**–**11** established the adenine N-3 addition to the unsubstituted cyclopropane carbon of duocarmycin A. This was first evident upon comparison of the ^1H NMR of **7** (Table II) with that of **2**–**5** (Table I, supplementary material).[10] Diagnostic of duocarmycin B_2 or C_2 and simple derivatives, the adduct **7** C10–H appeared as a single proton (1 H) at a characteristic chemical shift of 4.1–4.4 ppm versus 3.5–3.6 ppm (2 H) for duocarmycin B_1 or C_1, and the large geminal coupling constant (J = 19.5 Hz) characteristic

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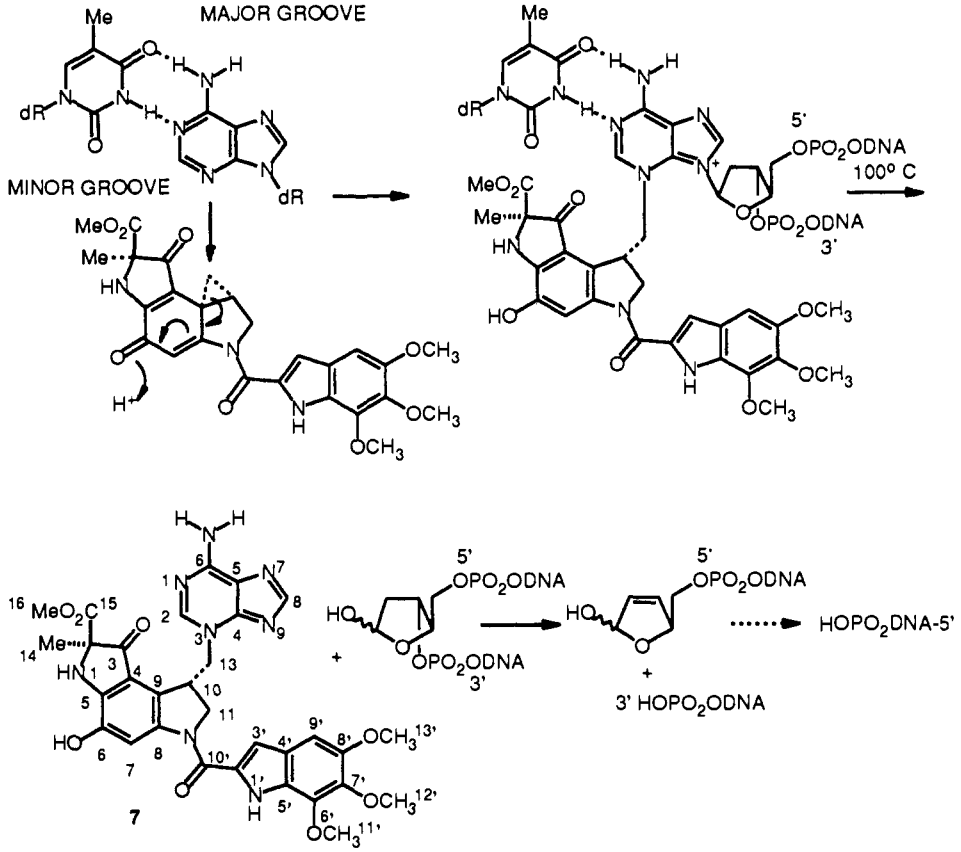
(16) Double-stranded calf thymus DNA (type I, Sigma) was manually shredded and subjected to sonication (12 h) in buffer to effect complete dissolution.

Scheme I. Duocarmycin A DNA Alkylation and Thermally Induced Depurination Reaction[a]



[a] The adenine N9 quaternized nitrogen constitutes one resonance form of the adenine N-3 alkylation and has been employed only to highlight the origin of the thermal labilization of the glycosidic bond.

Table II. ^{1}H NMR of **7** (600 MHz)

	CD_3OD	1% CF_3CO_2D–CD_3OD	DMF-d_7	15% CD_3OD-$CDCl_3$
N1′–H			11.21	
C6–OH			10.39	
Ade-C8–H (1 H, s)	8.30	8.33	8.20	7.71
Ade-C2–H (1 H, s)	8.02	8.02	7.88	7.64
C3′–H (1 H)	6.98	6.96	7.00	6.80
C7–H (1 H, s or br s)	7.26	7.22	7.26	7.85
C9′–H (1 H, s)	7.00	7.00	7.00	6.77
NH_2 (2 H, br s)	7.95		7.78	
N1–H (1 H, s)	5.19	5.10	5.15	
C11–H (1 H)	4.78	4.85[a]	4.86[a]	4.88[a]
	(dd, J = 13.5, 5.3 Hz)	(dd, J = 13.5, 4.8 Hz)	(dd, J = 13.5, 5.3 Hz)	(dd, J = 13.7, 4.2 Hz)
C13–H (1 H)	4.70	4.72[a]	4.97[a]	5.03[a]
	(d, J = 11 Hz)	(d, J = 11 Hz)	(d, J = 10 Hz)	(d, J = 11 Hz)
C13–H (1 H)	4.62	4.62[a]	4.56[a]	4.40[a]
	(dd, J = 11, 8.7 Hz)	(dd, J = 11, 9.8 Hz)	(dd, J = 10, 9.3 Hz)	(dd, J = 11, 9.3 Hz)
C11–H (1 H)	4.55	4.53[a]	4.70[a]	4.66[a]
	(dd, J = 13.5, 8.7 Hz)	(dd, J = 13.5, 8.7 Hz)	(dd, J = 13.5, 7.2 Hz)	(dd, J = 13.7, 6.0 Hz)
C10–H (1 H)	4.22 (m)	4.18 (m)	4.38 (m)	4.17 (m)
C11′–H (3 H, s)	4.08	4.6	4.03	4.00
C12′–H (3 H, s)	3.92	3.90	3.92	3.93
C13′–H (3 H, s)	3.92	3.90	3.89	3.90
C16–H (3 H, s)	3.63	3.63	3.68	3.77
C14–H (3 H, s)	1.46	1.44	1.60	1.70

[a] Tentative assignments based on the comparison of coupling constants derived from the spectrum recorded in CD_3OD and its assignments based on 2D H^1–H^1 NOESY (CD_3OD).

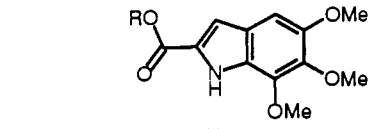
of **2**/**4** was not observed. Further consistent with the assignment were the chemical shifts and assigned coupling constants for C13–H (2 H) and C11–H (2 H). Even more revealing was the ^{13}C NMR of **7** (Table III) which was found to be in excellent agreement with that of duocarmycin B_2/C_2. The diagnostic ^{13}C NMR characteristics of **3**/**5** which distinguish them from **2**/**4** are found in the carbons within or proximal to the fused five- versus six-membered ring. The adduct **7** ^{13}C NMR proved consistent with that observed for **3**/**5**: C-8 at 137–138 versus 128–129 ppm, C-7 at 112–113 versus 117–118 ppm, C-10 at 41–43 versus 33–34 ppm, and C-4 at 119–121 versus 115–117 ppm. Moreover, the ^{13}C NMR chemical shift of C10 (41.2 ppm) proved comparable to that found in the CC-1065 adenine adduct **11** (40.1 ppm).[13]

The structure and ^{1}H NMR assignments of **7** were firmly established through interpretation of the 2D ^{1}H–^{1}H NOESY NMR spectrum which proved consistent only with alkylation of

Table III. ^{13}C NMR of **7** and Related Agents

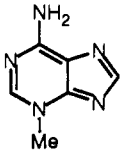
	7[a]	5[a]	5[c]	3[b]	4[c]	2[b]	11[d]	10[a]
C-3	199.4	197.5	196.6	196.6	197.8	196.8		
C-15	170.6	171.8	169.6	169.5	169.2	169.7		
C-10′	161.2	162.2	160.5	160.5	163.3	164.5		
C-8′	149.0	148.2	150.4	150.1	149.1	150.2		
C-6	151.2	151.8	150.1	150.4	152.2	151.7		
C-5	145.8	146.0	144.2	144.2	141.2	141.6		
C-7′	141.6		140.9	140.9	139.5	140.4		
C-6′	140.3		138.7	138.7	139.0	138.9		
C-8	138.2	138.0	137.7	137.6	128.4	128.9		
C-2′	131.4	131.9	129.1	129.1	130.7	129.1		
C-5′	127.1	127.8	126.0	126.0	125.4	126.1		
C-4′	125.1	125.5	123.5	123.5	122.7	123.1		
C-4	119.2	120.5	119.5	120.2	114.8	117.1		
C-9	116.1	116.0	115.6	115.6	114.7	116.6		
C-7	112.7	112.9	112.5	112.5	117.2	118.2		
C-3′	108.0	108.1	107.9	107.9	106.6	108.3		
C-9′	99.5	99.4	98.0	98.0	97.9	97.9		
C-2	72.0	72.1	71.2	71.2	70.1	71.1		
C-12′	62.0	61.9	61.5	61.5	60.9	61.5		
C-13′	61.8	61.7	61.2	61.2	61.0	61.2		
C-11′	56.6	56.8	56.4	56.4	55.9	56.3		
C-11	54.1	56.2	55.0	56.1	54.7	44.8	54.39	
C-16	53.4	53.5	53.4	53.4	52.6	53.4		
C-13	59.9	47.4	46.4	35.6			54.76	
C-10	41.2	43.3	42.3	42.0	32.9	33.9	40.14	
C-14	23.6	21.0	22.0	22.0	20.2	21.8		
C-12					51.2	53.0		
Ade-C2	152.4						153.1	152.9
Ade-C4	151.0						151.5	149.7
Ade-C5	122.3						121.5	119.5
Ade-C6	155.5						155.9	155.3
Ade-C8	148.0						144.4	145.9
N-CH_3								36.9

[a] MeOH-d_4. [b] $CDCl_3$.[6] [c] DMSO-d_6.[6] [d] DMF-d_7.[13]

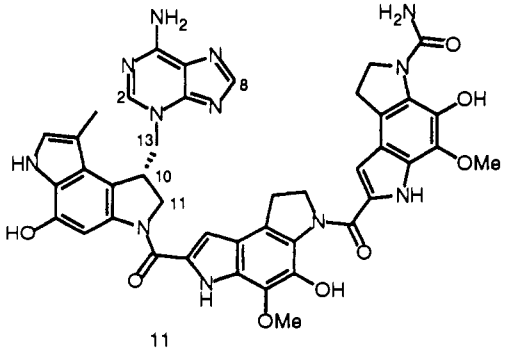


8 R = H

9 R = Me

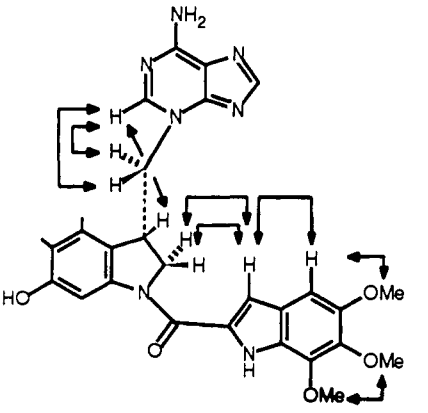


10



11

Figure 2.



Diagnostic NOE's

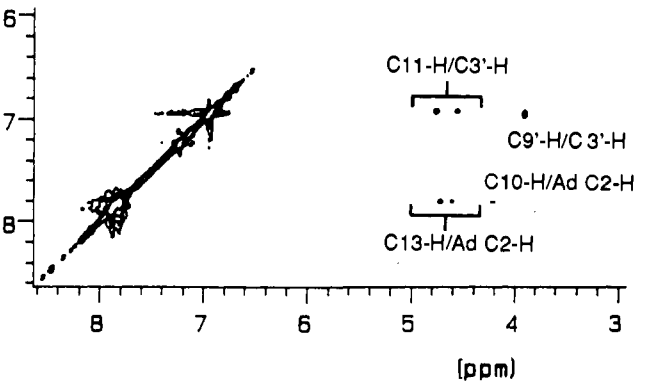


Figure 3. Contour plot of aromatic region of 2D ^1H–^1H NOESY spectrum of **7** (CD_3OD, 600 MHz).

the agent unsubstituted cyclopropane carbon by N-3 of adenine, Figures 2 and 3. The assigned chemical shifts for adenine C8–H (8.30 ppm) and adenine C2–H (8.02 ppm) were based on the observed NOE between C13–H and C10–H with adenine C2–H, and the assignments proved consistent with those found for 3-methyladenine (**10**, 8.24 and 7.90 ppm, respectively). Importantly, these observed NOEs served to unambiguously establish the C13–H ^1H NMR assignments. Similarly, a strong NOE between C11–H and C3′–H permitted the unambiguous assignment of C3′–H (6.98 ppm) and further distinguished C11–H from C13–H.

In addition, the lack of observation of an NOE between C11–H and adenine C2–H further ruled out the alternative adduct structure (no C12–H/adenine C2–H NOE). Additional diagnostic NOEs between C3′–H and C9′–H (7.00 ppm), C9′–H and C13′–H (3.92 ppm), and C12′–H and C11′–H (4.08 ppm) served to establish the respective assignments, Figure 2.

The isolation, characterization, and quantification of the thermally released adduct **7** derived from the duocarmycin alkylation of DNA unambiguously establishes the predominant (86–92%) DNA alkylation reaction for the agents as that which proceeds by adenine N-3 addition[9–17] (minor groove) to the unsubstituted cyclopropane carbon of duocarmycin A. Duocarmycin A and C_1–C_2 provide the same adduct **7** requiring that C_1 close to A prior to concurrent with the adenine N-3 alkylation. Consistent with these observations, the rate of alkylation by the three agents follow the order of A > C_2 > C_1.

Experimental Section[18]

Alkylation of DNA by Duocarmycin C_2 (5) and Isolation of Adduct 7. A solution of **5** (3.18 mg, 5.85 μmol) in *N,N*-dimethylformamide (1.6 mL) was treated with calf thymus DNA[16] (280 mg, 140 base-pair equiv) in 0.1 M sodium phosphate buffer (pH 7.4, 40 mL), and the mixture was stirred slowly for 7 days (23 °C) under argon in the dark. After precipitation of the DNA with the addition of 4 mL of 2 M aqueous sodium chloride and 100 mL of ethanol, 0.66 mg (21%) of **5** was recovered from the supernatant. The precipitated DNA was dissolved in 0.1 M sodium phosphate buffer (pH 7.4, 40 mL) and 1-butanol (40 mL), and the solution was warmed at 100 °C for 25 min with stirring. After cooling to 25 °C, the 1-butanol layer was separated. The aqueous layer was warmed with fresh 1-butanol (40 mL) for 25 min (100 °C). The combined 1-butanol layers were concentrated in vacuo, and the residue was purified by Sephadex LH-20 column chromatography (2 × 12 cm, 0–95% MeOH–water, gradient elution). The fractions displaying a pale yellow fluorescence under UV (TLC) were combined and lyophilized to afford the adduct **7** (2.74 mg, 73%, 92% based on recovered **5**) as a yellow solid.[19] The isolation of **7** (0.6–8.0 mg, 51–73%) from the DNA alkylation of **5** (1.0–10.0 mg, recovered **5**: 35–21%) was routinely conducted following this procedure (86–92% yield based on the consumption of **5**): mp 164–167 °C (dec); $[\alpha]^{23}_D$ −31° (*c* 0.26, MeOH), −45° (*c* 0.17, MeOH), −83° (*c* 0.084, MeOH), −124° (*c* 0.057, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) Table II; ^{13}C NMR (CD_3OD, 150 MHz) Table III; IR (KBr) ν_{max} 3752, 3332, 2926, 2852, 1736, 1648, 1618, 1500, 1412, 1308, 1228, 1164, 1110, 1046, 828 cm^{-1}; UV (MeOH) 340 (ε 28 000), 294 nm (27 000); CD (MeOH) $[\theta]^{23}$ 326 (+10 300), 266 (−22 100), 226 nm (−12 900); FABMS (DTT/DTE), *m/e* (rel intensity) 643 (M^+ + H, 75), 307 (70), 234 (base); FABHRMS (DTT/DTE), *m/e* 643.2278 ($C_{31}H_{30}N_8O_8$ + H^+ requires 643.2265).

The adduct **7** may be purified by chromatography on silica gel (SiO_2) albeit with some loss of material. This may be minimized by pretreating the SiO_2 with 5% Et_3N–CH_2Cl_2 and CH_2Cl_2 prior to chromatography (SiO_2, 2 × 15 cm, 0–5% MeOH–CH_2Cl_2 gradient elution).

The ^{1}H NMR phase-sensitive NOESY NMR spectrum (600 MHz) was run at 800 ms mixing time, 6.5 KHz spectral window, 158 ms aquisition time, 3.9 s recycle delay, 512 complex t_1 data points, and 24 scans per increment. The data were processed with a Gaussian function in t_2 and a combination of Gaussian and shifted sinebell functions in t_1. The sample was deoxygenated by ten freeze–pump–thaw cycles with argon purging.

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Registry No. 1, 118292-34-5; **2**, 124325-93-5; **3**, 124325-94-6; **4**, 118292-35-6; **5**, 118292-36-7; **6**, 130288-24-3; **7**, 134781-55-8; adenine, 73-24-5.

Supplementary Material Available: Table of ^{1}H NMR data of **7** and related agents (1 page). Ordering information is given on any current masthead page.

(17) Baker, B. F.; Dervan, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 2700.

(18) Proton nuclear magnetic resonance spectra (^{1}H NMR) and carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded on a Varian VXR-600, General Electric QE-300, or Varian Gemini 200 instrument, and chemical shifts are reported in parts per million relative to internal tetramethylsilane (0.00 ppm). Infrared spectra (IR) were recorded on a Perkin-Elmer 1800 Fourier transform spectrometer as potassium bromide pellets (solids). Ultraviolet spectra (UV) were recorded on a Hewlett-Packard 8451A diode array spectrophotometer. Electron-impact mass spectra (EIMS) and chemical ionization mass spectra (CIMS) were recorded on a Finnigan 4000 spectrometer. Fast atom bombardment mass spectra (FABMS) and high-resolution mass spectra (HRMS) were recorded on a Kratos MS-50 spectrometer. Flash chromatography was performed on 230–400 mesh silica gel. Analytical and preparative high-performance liquid chromatography (HPLC) was performed using a Gilson model 320 dual pump chromatograph equipped with an ISCO V^4 variable wavelength absorbance detector. CD spectra were recorded on a JASCO Model J-600 spectropolarimeter. *N,N*-Dimethylformamide (DMF) was distilled from powdered calcium hydride (CaH_2) under vacuum and stored under argon. All bulk extraction and chromatographic solvents [hexane, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), tetrahydrofuran (THF)] were distilled prior to use. Melting points (mp) were determined on a Thomas Hoover melting point apparatus and are uncorrected.

(19) For the preliminary characterization of **7**, see: ref 10 and Sugiyama, H.; Hosoda, M.; Saito, I.; Asai, A.; Saito, H. *Tetrahedron Lett.* **1990**, *31*, 7197.