in over 60% yield as 5:1 mixture of epimers. Because methoxy groups are known to have a cis-directing effect on the ene reaction of enol ethers,[11] the location of the double bond in **12** directly established the *Z* configuration of **11a**, which in turn allowed assignment of configuration **8a** to the "major" addition product formed from **7**.

On the basis of results reported by Asveld and Kellog[12] we could expect that by changing the reaction condition the introduction of a hydroperoxide function at C(3) of **11** and the formation of **13** would become possible. When the photooxygenation was carried out in methanol at −78 °C (solution of the sodium salt of **11a**), a complex mixture of products was formed. Although none of these could be identified, we assume that **13** was a major product. Indeed, when the crude mixture of oxygenation products was treated with acid (HCOOH, CH_2Cl_2, 0 °C, 24 h), crystalline qinghaosu (**1**) was obtained in 30% yield. Our synthetic material was identical (mp, $[\alpha]_D$, CD, NMR, IR) with an authentic sample of the natural product.[13]

Acknowledgment. We thank R. Burren, W. Haesler, and H. Zeller for technical assistance and the staff of the Central Research Department for the determination of physical and analytical data.

Registry No. 1, 63968-64-9; **2**, 89-79-2; **3**, 84051-29-6; **4**, 84051-30-9; **5**, 84051-31-0; **6**, 84051-32-1; **7**, 84051-33-2; **8a**, 84051-34-3; **8b**, 84064-31-3; **9**, 84051-35-4; **10**, 84051-36-5; **11a**, 84051-37-6; **11b**, 84064-32-4; **12** (isomer 1), 84051-38-7; **12** (isomer 2), 84064-33-5; **12**, 84051-39-8.

Supplementary Material Available: IR, ^1H NMR, melting point, and optical rotation data for key intermediates and final product (2 pages). Ordering information is given on any current masthead page.

(11) (a) Rousseau, G.; Le Perchec, P.; Conia, J. M. *Tetrahedron Lett.* **1977**, 2517–2520. (b) Rousseau, G.; Le Perchec, P.; Conia, J. M. *Synthesis* **1978**, 67–70. (c) Rousseau, G.; Lechevallier, A.; Huet, F.; Conia, J. M. *Tetrahedron Lett.* **1978**, 3287–3290. (d) Lerdal, D.; Foote, C. S. *Ibid.* **1978**, 3227–3230.

(12) Asveld, E. W. S.; Kellogg, R. M. *J. Am. Chem. Soc.* **1980**, *102*, 3644–3646.

(13) An authentic sample of qinghaosu was kindly provided by Dr. W. H. Wernsdorfer, WHO, Geneva.

Synthesis of the Cytotoxic Germacranolide Eucannabinolide

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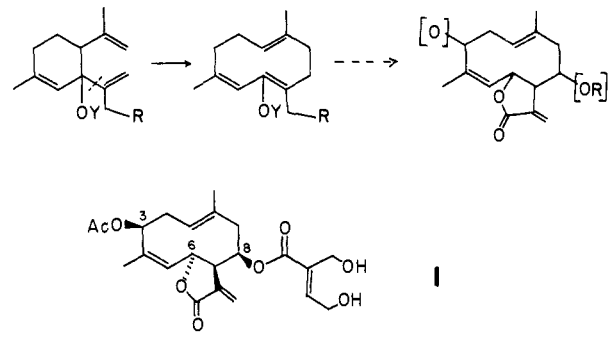
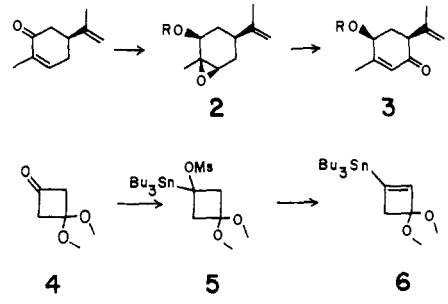
Received July 26, 1982

Previous results[1] from our laboratory have demonstrated that the oxy-Cope rearrangement provides a smooth pathway from monoterpenoid starting materials to appropriately functionalized germacrane-like intermediates as shown in Scheme I. Application of the route to germacranolide synthesis, however, is problematic because of the characteristic oxygenation at C8 which threatens β elimination after the ring expansion takes place. Stereochemical and regiochemical uncertainties are also present since the configurations at C6 and C7 (at least) and the direction of lactonization of the acrylic acid appendage would need to be set while on a conformationally flexible macrocyclic framework. Effective solutions to these problems have been found that allow rational construction of the germacranolide eucannabinolide (**1**).[2] An account of these solutions follows.

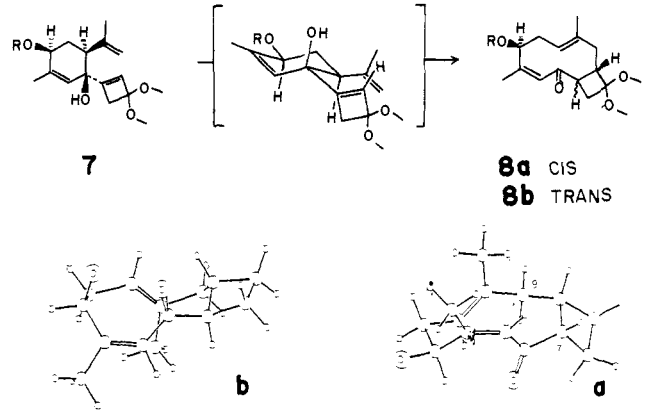
Scheme I



Scheme II



Scheme III



Our synthesis began with (+)-carvone. Reduction ($LiAlH_4$, Et_2O, 0 °C), epoxidation (MCPBA, CH_2Cl_2, 25 °C), and protection ($PhCH_2OCH_2Cl$, *i*-Pr_2NEt, 25 °C) yielded **2** in >70% yield (Scheme II).[3] The epoxide was eliminated via the selenoxide ((1) PhSeK–LiBr, THF, 25 °C; (2) 30% H_2O_2, $NaHCO_3$, NaOAc, THF, 60 °C, 16 h)[4] to a tertiary allylic alcohol, which was oxidized (Jones' reagent, 0 °C, 1.5 h) to the required enone **3** (53% yield from **2**).[5]

An appropriate equivalent of the required (alkoxyvinyl)acrylic acid appendage was found to be a cyclobutenone acetal which was prepared from the known acetal of the ketene/ethoxyacetylene

(1) (a) W. C. Still, *J. Am. Chem. Soc.*, **99**, 4186 (1977); (b) W. C. Still, *ibid.*, **101**, 2493 (1979). An application of the oxy-Cope route to the synthesis of 3-oxygenated germacranolides has been reported recently: C. Kuroda, H. Hirota, and T. Takahashi, *Chem. Lett.*, 249 (1982).

(2) M. J. Pettei, I. Miura, I. Kubo, and K. Nakanishi, *Heterocycles*, **11**, 471 (1978); T. Takahashi, H. Eto, T. Ichimura, and T. Murae, *Chem. Lett.*, 1345 (1978); F. Bohlmann, P. K. Mahanta, A. A. Natu, R. M. King, and H. Robinson, *Phytochemistry*, **17**, 471 (1978); W. Herz and S. V. Govindan, *ibid.*, **19**, 1234 (1980). Eucannabinolide is identical with schkuhrin I, hydroxychromolaenide, and hiyodori lactone A and has been proposed as the common name of **1**.

(3) Compounds were characterized by IR, 270-MHz ^1H NMR, and (in selected cases) mass spectra. Yields refer to isolated chromatographically pure compounds.

(4) K. B. Sharpless and R. F. Lauer, *J. Am. Chem. Soc.*, **95**, 2697 (1973).

(5) Alternative direct oxidation (e.g., CrO_3–dimethylpyrazole, CH_2Cl_2: W. G. Salmond, M. A. Barta, and J. L. Havens, *J. Org. Chem.*, **43**, 2057 (1978)) of carveol acetate to **3** was possible although the yield was only 10–20% and the procedures were not convenient for large-scale reactions.

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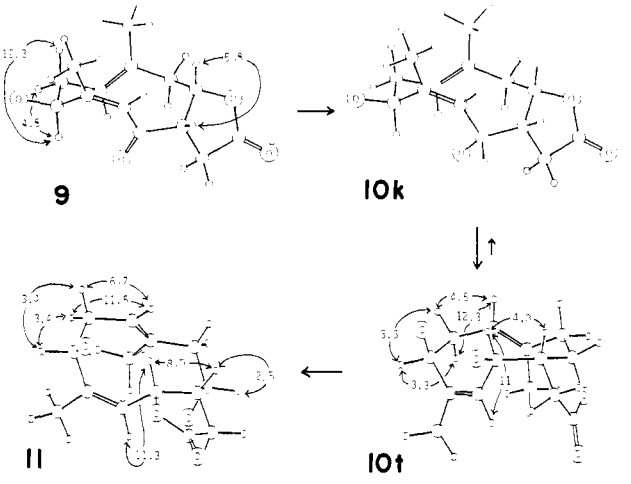
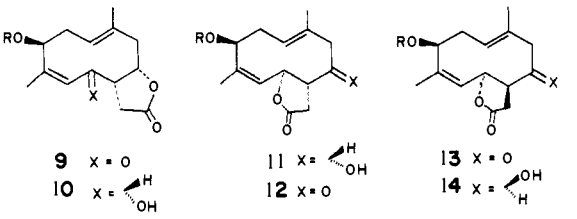
Scheme IV



cycloadduct (**4**).[6] Low-temperature addition of $Bu_3SnMgCl$ (Bu_3SnLi,[7] $MgCl_2$, THF, −70 °C, 5 min) followed by direct in situ mesylation (1.2 equiv of MsCl) gave **5** in 68% yield. Elimination was accomplished with excess powdered K_2CO_3 in Me_2SO (100 °C, 1 h) and led to the desired cyclobutenyl tin reagent **6** (96% yield).[8]

Coupling of **3** and **6** proceeded via lithiation of **6** (1.3 equiv of **6**, 1.0 equiv of *n*-BuLi, THF, −70 °C 30 min) and addition of 1.0 equiv of the enone **3**. The adduct **7** (Scheme III), that formed was as a single diastereomer (in the dimethyl acetal series) to the extent of at least 6:1 and was isolated by flash chromatograpohy in 82% yield (85% conversion). Oxy-Cope ring expansion was effected by using 5 equiv of $KN(TMS)_2$ in dimethoxyethane at 85 °C (14 h) and led to formation of **8** in high yield. Assuming that the cyclobutenyllithium adds trans to the bulky isopropenyl substitutent and that the oxy-Cope rearrangement proceeds via a chairlike transition state, then the stereochemistry of the C3 and the C8 substituents must be trans as shown above. The C7 stereochemistry is the result of a kinetic protonation step and turned out to be nearly a 1:1 mixture of diastereomers **8a** and **8b** under a variety of protonation conditions. It was found, however, that when the mixture was stirred in dry MeOH containing powdered K_2CO_3 (25 °C, 24 h), a 15:1 ratio (270-MHz ^1H NMR and HPLC) of isomers was produced. The major isomer was tentatively assigned as cis-**8a**, and its isolated yield based on **7** was 90% at 80% conversion. At this point, our cis assignment rested largely on an MM2 molecular mechanics[9,11] evaluation of the most stable conformations of **8a** and **8b**. The MM2 force field places **8a** approximately 3 kcal more stable than **8b** and furthermore shows in **8a** a nicely aligned array of atoms suitably arranged for a long-range W-type coupling between the C7 hydrogen and the equatorial C9 hydrogen. Such coupling was displayed in the 250-MHz ^1H NMR as a 4.5-Hz doublet.

Chart I

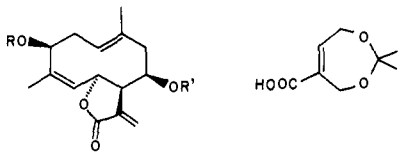


With homogeneous **8a** in hand, the butyrolactone moiety was demasked by gentle acid hydrolysis (aqueous HOOCCOOH/silica gel, CH_2Cl_2, 35 °C, 2 h) and Baeyer–Villiger oxidation (anhydrous H_2O_2, Ti(O-*i*-Pr)$_4$, *i*-Pr_2NEt, Et_2O, −30 °C, 15 min).[10] The resulting ketolactone **9** (Scheme IV) was not purified but was immediately reduced with sodium borohydride (MeOH, 0 °C, 30 min) to yield **10** (55% yield based on **8a**) as the only isolable hydroxylacetone. This reduction is an interesting one with respect to the lowest energy conformations of the species involved. The MM2 ground-state structure of **9** is shown above and is compatible with observed ^1H NMR coupling constants for an axial C3–H (dd, J = 12.3, 4.5 Hz) and the C7–H–C8–H splitting (J = 5.8 Hz). A plausible mechanism for the formation of **10** involves peripheral addition of hydride to a low-energy conformation of **9**. This addition would lead kinetically to a relatively strained conformation (**10k**) having the hydroxyl pushed over the center of the ring. Conformational equilibration finally leads to the ground-state structure (**10t**), which is predicted by the MM2 force field to have exchanged the transannular C6–OH interaction for an energetically less demanding axial C3–OH. Again, the ^1H NMR is consistent with the **10t** geometry as indicated by the coupling constants shown above.

In order to convert **10** to the natural ring substitution and stereochemistry, both the direction of lactonization and the configurations at C7 and C8 had to be changed. Mechanisms for these adjustments were suggested by MM2 calculations which predicted the C6-lactone **11** (Chart I, Scheme IV) to be 1 kcal more stable than **10** and which also predicted the trans-ketolactone **13** to be of energy similar to **12**.[11] Guided by this information, we treated **10** with catalytic K_2CO_3 in MeOH (25 °C, 5 h) to yield the sensitive isomeric lactone **11** in 61% which Equilibration in CD_3OD gave an equilibrium ratio of 9:1 as judged by ^1H NMR. Collins oxidation (CH_2Cl_2, 25 °C, 5 min) then gave **12** (71%), which was equilibrated with DBU in THF (25 °C, 3 h). The equilibrium ratio was found to be 7:1 by ^1H NMR and the pure trans-isomer **13** was isolated by flash chromatography in 70% yield. Peripheral reduction ($NaBH_4$, MeOH) gave **14** as the only product (93%), which should possess the natural regiochemistry and stereochemistry. The C3 benzyloxymethyl protecting group was removed catalytically (H_2–20% $Pd(OH)_2$/C, 97% EtOH, 25 °C, 22 psi) to give a crystalline diol (**15**, 78%, mp 146–147 °C), which was subjected to X-ray crystallographic analysis.[12] The expected structure was confirmed and is shown in the supplementary data.

The synthesis was completed by means of straightforward conversions. Thus silylation ((trimethylsilyl)imidazole, C_5H_5N, CH_2Cl_2, 25 °C) and hydroxymethylation ((a) LDA, THF; (b) HCHO(g), −70 °C) gave the aldol adduct (75% at 77% conversion). Mesylation (MsCl, Et_3N, (dimethylamino)pyridine, CH_2Cl_2, 25 °C) followed by elimination (DBU, dioxane, 70 °C, 30 min) formed the desired methylene lactone **16** (R, R′ = Me_3Si, 82% yield). Desilylation (Bu_4NF, THF, 25 °C) and acetylation of the more reactive C3 hydroxyl (AcOH, DCC, 4-pyrrolidinopyridine) gave **16** (R = Ac, R′ = H; mp 122–123 °C, 82% yield), which was finally esterified (DCC, 4-pyrrolidinopyridine, 52%) at C8 with the dihydroxytiglic acid acetonide **17**[13] and deprotected

(6) H. H. Wasserman, J. U. Piper, and E. V. Dehmlow, *J. Org. Chem.*, **38**, 1451 (1973).
(7) W. C. Still, *J. Am. Chem. Soc.*, **100**, 1481 (1978).
(8) For preparative purposes, the mixed methyl ethyl acetals of **4–8** turned out to be most easily handled although the dimethyl acetals simplified spectral assignments. Thus yields are reported for the methyl ethyl acetal series while accurate diastereomer ratios refer to the dimethyl acetal series. Although minor ratio differences may have occurred, careful spectral comparisons indicate that any such differences are small.
(9) Reviews: N. L. Allinger, *Adv. Phys. Org. Chem.*, **13**, 1 (1976); D. B. Boyd and K. B. Lipkowitz, *J. Chem. Educ.*, **59**, 269 (1982). The MM2 program was obtained from Indiana University's Quantum Chemistry Program Exchange as program no. 395. For other recent applications of molecular mechanics to synthesis see W. C. Still and I. Galynker, *Tetrahedron*, **37**, 3981 (1981); *J. Am. Chem. Soc.*, **104**, 1774 (1982).
(10) Alternative Baeyer–Villiger reaction with 30% H_2O_2/Na_2HPO_4 in MeOH also gave the desired lactone but in lower yield.
(11) These strain-energy differences refer to the lowest energy conformations found. Initial geometries were produced by a ring-generating computer program operating at 15° dihedral angle resolution, which formed all possible rings having reasonable ring closure parameters (typical closure distances = 1–2 Å and bond angles = 90–140°). Due to the approximate nature of molecular mechanics calculations on complex systems, energy differences of less than 1 kcal/mol have little predictive value.
(12) We thank Professor S. J. Lippard for his assistance in the determination of this structure. Detailed crystallographic results will be reported elsewhere.



16 17

($C_5H_5NH^+OTs^-$, MeOH, $HOCH_2CH_2OH$, 54%) to yield **1**. Synthetic eucannabinolide thus prepared was found by IR, ^1H NMR (270 MHz), TLC, and CD to be identical with a sample of authentic **1** kindly supplied by Professor Koji Nakanishi here at Columbia.[14]

Registry No. 1, 38458-58-1; **2**, 84066-29-5; **3**, 84066-30-8; **4**, 38425-58-0; **5**, 84066-31-9; **6**, 84066-32-0; **7**, 84066-33-1; **8a**, 84066-34-2; **8b**, 84107-75-5; **9**, 84066-35-3; **10**, 84066-36-4; **11**, 84142-53-0; **12**, 84066-37-5; **13**, 84066-38-6; **14**, 84066-39-7; **15**, 84066-40-0; **16** (R, R′ = TMS), 84066-41-1; **16** (R = Ac; R′ = H), 84066-42-2; **17**, 84066-43-3; (+)-carvone, 2244-16-8.

Supplementary Material Available: Infrared and 250- or 270-MHz proton NMR spectra for all numbered compounds, X-ray structure of **15** shown as an ORTEP stereopair, and molecular mechanics structures and energies for compounds **8–13** are included (22 pages). Ordering information is given on any current masthead page.

(13) This compound was prepared from methyl tiglate as follows: (1) NBS, CCl_4, cat. $(PhCOO)_2$ (48%); (2) NaOAc, Ac_2O (72%); (3) LiOMe, MeOH (56%); (4) $CH_3C(OCH_3)=CH_2$, cat. $C_5H_5NH^+OTs^-$, CH_2Cl_2 (88%); (5) (a) NaOH, H_2O, (b) pH 2.5 (76%).

(14) This work was supported by Grand No. CA23094, awarded by the National Cancer Institute, DHEW.

Effect of Intercalating Drugs and Temperature on the Association of Sodium Ions with DNA: Sodium-23 NMR Studies

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Received August 2, 1982

The conformations and physical properties of many biological polymers in aqueous solutions are strongly influenced by small counterions.[1-5] Record and co-workers have shown that cationic peptides and proteins that bind strongly at specific sites on DNA release an equivalent amount of associated counterion.[1,3,6] We have demonstrated similar effects with the intercalators quinacrine[7,8] and ethidium.[8,9] Sodium-23 NMR spectroscopy has been useful in studies of synthetic and natural polymers.[10] Record and co-workers have examined association of sodium and other simple counterions with DNA by using ^{23}Na line widths.[11,12] In the work reported here we have, for the first time, monitored sodium ion release from DNA as a result of binding of an intercalating drug, ethidium bromide, to DNA, observed sodium ion release from native DNA on denaturation, and by varying the charge density on DNA, obtained information on the relaxation of $^{23}Na^+$ associated with DNA. The ability to vary the charge density on DNA in a quantitative manner is especially significant because it allows direct comparison of ^{23}Na ion relaxation when associated with DNA to predictions from polyelectrolyte theory. With synthetic polyanions it has been shown both theoretically and experimentally that the $^{23}Na^+$ relaxation rate has a quadratic dependence on the degree of neutralization (α) above α values of approximately 0.3, which is the range of ion condensation.[10,12-14] Since the charge density of native DNA cannot be significantly varied in a pH titration, we show in this work that titration of DNA with cationic intercalators can be particularly useful in evaluation of relaxation mechanisms.

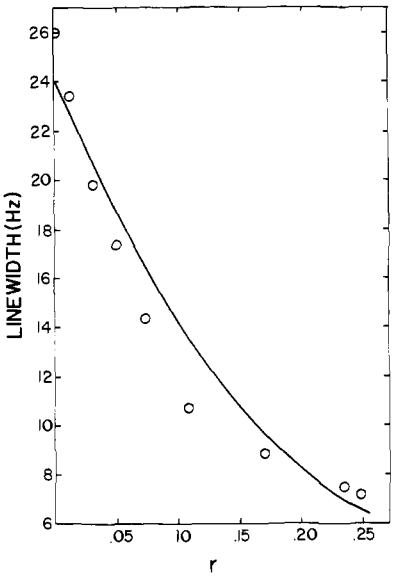

Figure 1. ^{23}Na NMR titration of DNA with ethidium bromide at 25 °C. The ^{23}Na ion line width is plotted vs. r, the ratio of moles of ethidium bound per mole of DNA phosphate. The open circles are experimental points, and the solid line was calculated as indicated in the text.

The binding of intercalators to DNA, unlike simple counterions, affects the charge density of the double helix in two potential ways: (i) the charge on the intercalator neutralizes some of the anionic charge of DNA, and (ii) insertion of the aromatic ring of the intercalator between base pairs of DNA increases both the local and the average phosphate to phosphate distances and, therefore, also decreases the DNA charge density.[7] In agreement with this prediction, the intercalation binding constant has been shown to depend on the counterion concentration,[7-9] indicating that, in the thermodynamic measurements, the total associated counterion is approximately linearly dependent on the amount of intercalator bound to DNA. It should be emphasized that the total amount of sodium ion associated with the double helix in the thermodynamic sense may be different from that monitored by the ^{23}Na NMR method. In Figure 1 results of ^{23}Na line width measurements in the presence of DNA at varying ratios of ethidium bromide to DNA phosphate are shown.[15] As a first approach

(1) (a) Record, M. T., Jr.; Anderson, C. F.; Lohman, T. M. *Q. Rev. Biophys.* **1978**, *11*, 103. (b) Manning G. S. *Ibid.* **1978**, *11*, 179.
(2) Forsen, S.; Lindman, B. *Methods Biochem. Anal.* **1981**, *27*, 289.
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(8) Wilson, W. D.; Jones, R. L. *Adv. Pharmacol. Chemother.* **1981**, *18*, 177.
(9) Gable, D.; Jones, R. L.; Wilson, W. D., unpublished results.
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(14) Kielman, H. S.; van der Hoeven, J. M. A. M.; Leyte, J. C. *Biophys. Chem.* **1976**, *4*, 103.

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