evaporated. The residue was crystallized from chloroform–hexane to give colorless prisms of **4** (4 mg), mp 266–269° dec, characterized by melting point, mixture melting point, tlc, and ir spectral comparison with **4** prepared from hellebrin.

Acetylation of Hellebrigenin.—A solution of hellebrigenin (510 mg) and *p*-toluenesulfonic acid (10 mg) in a mixture of chloroform (5 ml) and isopropenyl acetate (10 ml) was stirred for 3 hr at 45–50°. The solution was evaporated and the residue was chromatographed on silicic acid (40 g). Elution with 0.5% methanol in chloroform gave a fraction (32 mg) rich in **2**. Elution with 1% methanol in chloroform gave a fraction (333 mg) rich in **1**. The latter fraction was recycled, and the product again was chromatographed on silicic acid to yield fractions (44 mg and 245 mg) rich in **2** and **1**, respectively. A second recycling of the 245-mg fraction yielded a further fraction (45 mg) rich in **2**. The combined fractions rich in **2** (121 mg) were chromatographed on silica gel (20 g). Elution with acetone–methanol–chloroform (10:2:88) yielded a fraction (99 mg) which was crystallized from methanol–ether as colorless prisms of hellebrigenin 3,5-diacetate (**2**, 30 mg), mp 209–214°. The melting point of this compound was not depressed by admixture with the sample from *B. abyssinica*. The two samples showed identical R_f values on tlc and gave superimposable ir spectra ($CHCl_3$).

Solvolysis of Hellebrigenin 3-Acetate.—A solution of hellebrigenin 3-acetate (**1**, 10 mg) in 20% aqueous methanol (5 ml) was treated with triethylamine (50 mg). After standing at room temperature for 16 hr, the solution was concentrated under reduced pressure to a small volume, diluted with water (5 ml), and extracted with chloroform (20 ml). The chloroform extracts were dried (Na_2SO_4) and the solvent was evaporated to yield an essentially homogeneous (tlc) colorless oil (9 mg). Crystallization from acetone–ether yielded colorless prisms of hellebrigenin, mp 225–227°, characterized by mixture melting point and tlc.

Attempted Solvolysis of Hellebrigenin 3,5-Diacetate.—A solution of hellebrigenin 3,5-diacetate (**2**, 9 mg) in 20% aqueous methanol (5 ml) was treated with triethylamine (50 mg) in the same manner as described for **1**, to yield an essentially homogeneous (tlc) colorless residue (9 mg). Crystallization from methanol–ether led to recovery of hellebrigenin 3,5-diacetate as colorless prisms, mp 214–217°.

Methyl Isohellebrigeninate (5).—Hellebrigenin (110 mg) was treated with a solution of sodium hydroxide (250 mg) in methanol (10 ml). After standing for 4 hr at room temperature, the solution was treated with ice–water (30 ml) and acidified with hydrochloric acid. The precipitate was filtered, washed with water, and dried under reduced pressure. The residue (86 mg) was crystallized from acetone–hexane to give prisms (64 mg). Recrystallization from methanol gave colorless prisms of **5**: mp 235–240° dec; uv λ_{max}^{MeOH} 300 mμ (ε 21,100); ir λ_{max}^{KBr} 2.96, 3.39, 3.48, 3.63, 5.81, 5.86, 6.19, 6.22, and 8.59 μ.

Anal. Calcd for $C_{25}H_{34}O_7$: C, 69.74; H, 7.96. Found: C, 70.06; H, 7.88.

Methyl Isohellebrigeninate 3,5-Diacetate (6).—Hellebrigenin 3,5-diacetate (50 mg) was treated with a solution of sodium hydroxide (40 mg) in methanol (4 ml). After 1 hr, the product was worked up as described for methyl isohellebrigeninate and the residue (35 mg) was chromatographed on silicic acid (5 g). The fractions eluted with chloroform were combined on the basis of tlc to give an oil (15 mg). Crystallization from ether–hexane gave colorless plates of **6** (8.5 mg): mp 207–210°; uv λ_{max}^{MeOH} 300 mμ (ε 24,800); ir λ_{max}^{KBr} 2.90, 3.37, 3.48, 3.63, 5.76, 5.81, 5.85, 6.19, 6.21, 7.95, and 8.60 μ.

Anal. Calcd for $C_{29}H_{38}O_8$: mol wt, 514.25668. Found: mol wt, 514.25830 (mass spectrum).

Registry No.—**1**, 4064-09-9; **2**, 16808-82-5; **4**, 21887-06-9; **5**, 21887-07-0; **6**, 21904-42-7.

Tumor Inhibitors. XLV.[1a] Crotepoxide, a Novel Cyclohexane Diepoxide Tumor Inhibitor from *Croton macrostachys*[1b]

S. MORRIS KUPCHAN,[2] RICHARD J. HEMINGWAY, AND ROGER M. SMITH

Department of Pharmaceutical Chemistry, University of Wisconsin, Madison, Wisconsin 53706

Received June 30, 1969

An alcoholic extract of the fruits of *Croton macrostachys* Hochst. *ex* A. Rich. was found to show significant inhibitory activity against the Lewis lung carcinoma in mice (LL). The major active component, crotepoxide (**1**), $C_{18}H_{18}O_8$, was isolated and found to be a novel cyclohexane diepoxide. Alkaline hydrolysis and spectral evidence showed it to be the benzoate and diacetate derivative of the triol **2**. Hydrogenation of **1** reduced the aromatic ring to give a hexahydro derivative **3**. On treatment with hydrochloric acid, crotepoxide yielded initially the monochlorohydrin **4**, and then the dideacetyldichlorohydrin **5**. Treatment of **1** with hydriodic acid yielded the iodohydrin **8** and the ene diol **9**, whose reactivity has been studied. The structure and absolute stereochemistry of **8** were established by X-ray crystallographic analysis and, together with spectral evidence, were used to confirm the absolute stereochemistry and structure of **1**.

In the course of a continuing search for tumor inhibitors from plant sources, an alcoholic extract of the fruits of *Croton macrostachys* Hochst. *ex* A. Rich. (Euphorbiaceae)[3] was found to show significant inhibitory activity against Lewis lung carcinoma carried in mice (LL).[4] We report herein the systematic fractionation of the active extract of *C. macrostachys* and the isolation and structural elucidation of crotepoxide, the major active principle.

The dried ground fruits of *C. macrostachys* were extracted continuously with ethanol for 16 hr. Partition of the concentrated ethanolic extract (A) between 10% aqueous methanol and petroleum ether resulted in concentration of the activity in the methanol phase (C). The residue after evaporation of the methanol was partitioned between 1-butanol (D) and water (E) (Chart I and Table I). Fraction D in chloroform was chromatographed on silicic acid. The active component was eluted with chloroform, and rechromatography on silicic acid led to the isolation of crotepoxide, which showed significant tumor-inhibitory activity against Lewis lung carcinoma in mice (LL) and Walker intramuscular carcinosarcoma in rats (WM)[4] (Table I).

On the basis of elemental analysis, crotepoxide was assigned the molecular formula $C_{18}H_{18}O_8$. The pres-

(1) (a) Part XLIV: S. M. Kupchan, R. J. Hemingway, and J. C. Hemingway, *J. Org. Chem.*, **34**, 3894 (1969). (b) This investigation was supported by grants from the National Cancer Institute (CA-04500) and the American Cancer Society (T-275), and a contract with Chemotherapy, National Cancer Institute, National Institutes of Health (PH 43-64-551).

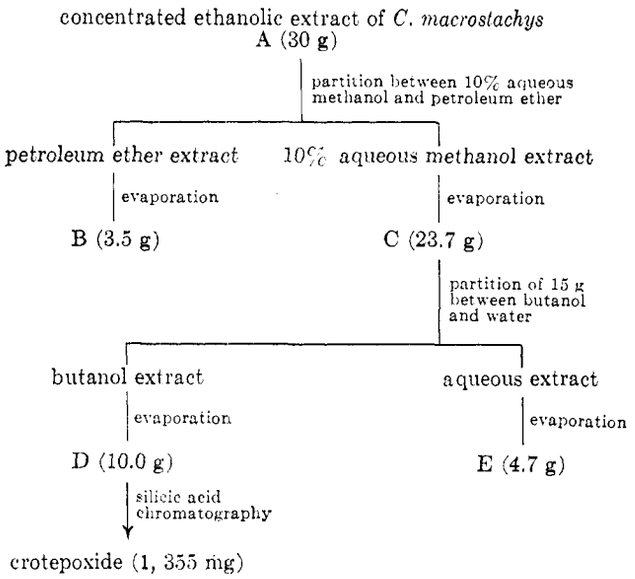
(2) Author to whom inquiries should be directed: Department of Chemistry, University of Virginia, Charlottesville, Va. 22901.

(3) Fruits gathered in Ethiopia in March 1965. The authors acknowledge the receipt of the dried plant material from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture (USDA), Beltsville, Md., in accordance with the program developed with the USDA by the Cancer Chemotherapy National Service Center (CCNCS).

(4) Assays were performed under the auspices of the CCNSC. The procedures were those described in *Cancer Chemotherapy Rept.*, **25**, 1 (1962).

CHART I

FRACTIONATION OF TUMOR-INHIBITORY EXTRACT FROM *C. macrostachys*



ence of a characteristic aromatic ultraviolet absorption at λ_{max} 274 and 281 mμ, of bands at 5.78, 6.24, and 9.00 μ in the infrared spectrum, and of a complex five-proton multiplet at τ 1.8–2.8 in the nmr spectrum suggested a benzoate ester. Further bands in the infrared spectrum at 5.71 and 8.20 μ and two singlets at τ 7.88 and 7.95 in the nmr spectrum suggested also the presence of two acetate groups. Under vigorous alkaline hydrolysis conditions, benzoic acid was the only product isolated, but under milder conditions, a polar neutral material, $C_7H_{10}O_5$, was isolated. This compound showed no ultraviolet absorption spectrum and no ester bands in its infrared spectrum, thus confirming the presence in crotepoxide of a benzoate and two acetate ester groups.

Hydrogenation of crotepoxide with platinum as a catalyst led to a hexahydro derivative, $C_{18}H_{24}O_8$, whose ultraviolet spectrum lacked aromatic absorption bands. The nmr spectrum of the compound, which was otherwise similar to that of crotepoxide, lacked the signals from the aromatic protons. Instead the spectrum contained an 11-proton multiplet at τ 8.0–9.0, typical of a cyclohexyl group. These results, which excluded the presence of other double bonds, and the molecular formula led to the conclusion that the alcohol moiety of crotepoxide was tricyclic. The absence of hydroxyl absorption bands from the infrared spectrum of crotepoxide indicated that the compound contained two ether linkages as well as the three ester groups.

The remaining signals in the nmr spectrum of crotepoxide could all be assigned to protons on carbons carrying oxygen (Table II). A study of the coupling constants revealed that these protons consisted of five methine groups linked in a continuous chain and two isolated protons, whose signals appeared as an AB quartet (J_{AB} = 12.0 Hz). As the position of this AB quartet was affected by the reduction of the benzoyl group, the signal could be assigned to a methylene group carrying the benzoate ester adjacent to a quaternary asymmetrical center. The chemical shifts of the methine protons suggested that two protons [τ 4.27 (d, J = 9.0 Hz) and 5.02 (dd, J = 9.0 and 1.5 Hz)], which from their coupling constants are adjacent at the end of the chain, were on carbons which carried the acetoxyl groups. The signals of these protons appeared at higher field as part of a complex multiplet, τ 5.4–6.7, in the spectrum of the hydrolysis product. Thus the three remaining protons, corresponding to the signals at τ 6.90, 6.56, and 6.32, must have been on carbons which carried the ether-linked oxygens.

TABLE I

ACTIVITY OF FRACTIONS FROM *C. macrostachys* IN *in vivo* ASSAY[a]

Fraction	Tumor system	Dose, mg/kg	Survivors	Animal wt change difference, g T − C	Tumor wt, mg T/C	T/C × 100
A	LL	500	0/4	...	...	...
		250	3/4	−3.8	222/1101	20
		125	3/4	−3.5	417/1101	37
B	LL	200	1/4	−7.6	...	...
		100	4/4	−3.2	575/885	64
		50	4/4	−1.3	644/885	72
C	LL	500	4/4	−4.1	222/885	25
		250	4/4	−1.8	445/885	50
		125	4/4	−2.7	569/885	64
D	LL	300	0/4	...	...	...
		150	2/4	−6.9	238/1302	...
		75	4/4	−3.1	614/1302	47
E	LL	360	4/4	−2.0	571/1302	43
		180	4/4	−2.6	623/1302	47
		90	4/4	−1.7	936/1302	71
1	LL	450	0/6	...	...	...
		300	1/6	+0.5	130/1373	...
		200	4/6	−1.8	496/1373	36
1	WM	450	4/4	−31	800/3600	22
		300	4/4	−30	1200/3600	33
		200	4/4	−19	1800/3600	50

[a] T, treated animals; C, control animals.

The spectral evidence led to formulation of the structures **1**, **2**, and **3** (apart from stereochemistry) for crotepoxide and its hydrolysis and hydrogenation products, respectively. Alternative structures, with 1,4 and 5,6 ether links, were excluded by later chemical work. Unsuccessful attempts were made to reduce the diepoxide system to a diene by the method of Cornforth, *et al.*[5]

In order to study the position and stereochemistry of the epoxides, crotepoxide (**1**) was treated with methanolic hydrochloric acid to yield under mild conditions monochlorohydrin **4**, $C_{18}H_{19}ClO_8$, and under more drastic conditions dideacetyldichlorohydrin **5**, $C_{14}H_{16}Cl_2O_6$.

The structure of **4** was deduced from comparison of its nmr spectrum with that of crotepoxide. The signal for the C-4 proton (τ 6.23) now appeared at slightly lower field, but that for the C-5 proton (τ 5.81) showed a larger shift to lower field, indicative that the chlorine atom had been introduced at C-5. In the spectrum of **5**, the signals for the C-7 protons were again present as an AB quartet, confirming the position of the benzoate ester at C-7, whereas the deacetylation had caused the C-2 and C-3 proton signals to appear at higher field, as part of a complex multiplet at τ 5.4–5.9. Mild acetylation of **5** yielded the triacetate **6**, $C_{20}H_{22}Cl_2O_9$. The nmr spectrum of **6** in DMSO-d_6 or

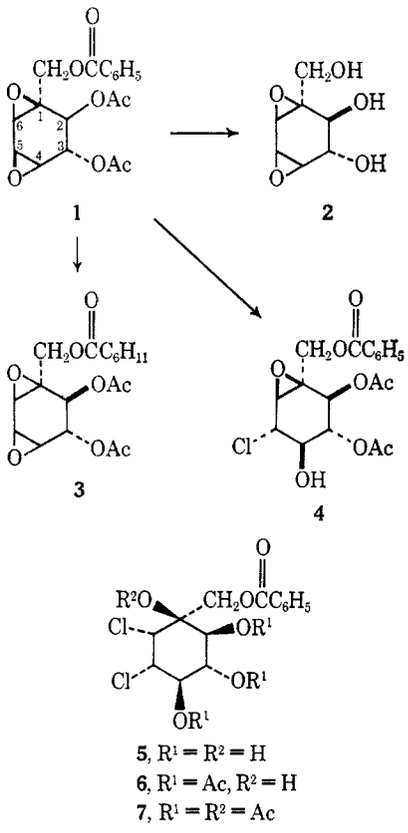
(5) J. W. Cornforth, R. H. Cornforth, and K. K. Mathew, *J. Chem. Soc.*, 112 (1959).

TABLE II

NUCLEAR MAGNETIC RESONANCE DATA FOR CROTEPOXIDE AND DERIVATIVES[a]

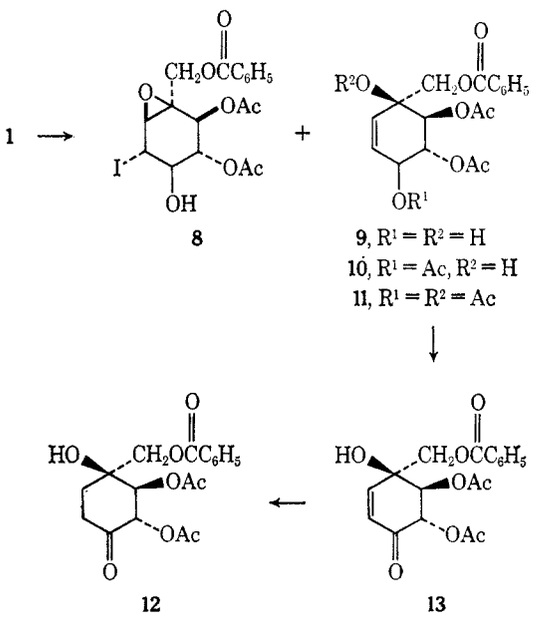
Compd[b]	C-2	C-3	C-4	C-5	C-6	C-7	-OCOCH$_3$	-OH
1	4.27 d (9.0)	5.02 dd (9.0, 1.5)	6.90 dd (1.5, 4.0)	6.56 dd (4.0, 2.5)	6.32 d (2.5)	5.42 d, 5.75 d (12.0)	7.88 s, 7.95 s	...
2[c]	C-2 to C-7, τ 5.4–6.7 m (7 H)						...	3.3 (3 H)
3[d]	4.38 d (9.0)	5.10 dd (9.0, 1.5)	6.93 dd (1.5, 4.0)	6.58 dd (4.0, 2.5)	6.45 d (2.5)	5.60 d, 6.13 d (12.0)	7.90 s, 7.93 s	...
4	4.27 d (8.5)	4.95 dd (8.5, 9.0)	6.23 dd (9.0, 8.0)	5.81 d (8.0)	6.38 s	5.42 d, 5.75 d (12.0)	7.89 s, 7.92 s	7.3 (1 H)
5	C-2 to C-6, τ 5.4 m (2 H) and 5.9 m (3 H)					5.00 d, 5.43 d (12.0)	...	4.0 (4 H)
6	C-2, C-3, and C-4, τ 4.5 m (3 H)			C-5 and C-6, τ 5.4 m (2 H)		5.29 d, 5.55 d (12.0)	7.92 s (6 H) 8.03 s	...
7	C-2 to C-6, τ 5.5 m (1 H) and 4.0–4.7 m (4 H)					4.95 d, 5.12 d (12.0)	7.75 s, 7.90 s 7.98 s, 8.05 s	...
8	4.24 d (8.5)	4.92 t (8.5)	6.05 t (8.5) 5.6 m	5.74 d (8.5)	6.05 s	5.43 d, 5.76 d (12.5) 5.54 d, 5.87 d (12.0)	7.89 s, 7.94 s 7.90 s (6 H)	7.30 s (1 H) 6.5–6.8 (2 H)
9	C-2, C-3, C-5, and C-6, τ 3.9–4.6 m (4 H)							
10	C-2 to C-6 τ 4.1–4.5 m (5 H)					5.52 d, 5.87 d (12.0)	7.92 s, 7.94 s, 7.97 s	7.4 (1 H)
11	C-2 to C-6 τ 3.5–4.5 m (5 H)					5.00 d, 5.60 d (11.0)	7.86 s, 7.94 s 7.95 s, 7.97 s	...
12	4.36 d (11.0)	4.57 d (11.0)	...	6.8–8.2 m (4 H)		5.60 d, 5.77 d (12.0)	7.86 s, 7.88 s	6.9 (1 H)
13	4.21 d (10.5)	4.35 d (10.5)	...	3.76 d (11.0)	3.05 d (11.0)	5.46 d, 5.76 d (11.0)	7.86 s, 7.88 s	7.0 (1 H)

[a] Spectra were determined on a Varian A-60A spectrometer using deuteriochloroform solutions unless otherwise stated. Values are given in τ units relative to tetramethylsilane as internal standard. Multiplicity of signals is designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet. Numbers in parentheses denote coupling constants in hertz. [b] All spectra except those of **2** and **3** contained a five-proton multiplet at τ 1.8–2.8 due to the aromatic protons. [c] Solvent pyridine-d_5. [d] τ 8.0–9.0 br m (11 H).



acetone-d_6 contained a singlet indicative of a D_2O-exchangeable proton and suggested that the hydroxyl, formed by the opening of the 1,6-epoxide, was tertiary. This hydroxyl group was also resistant to oxidation with Jones reagent but on treatment under forcing conditions with isopropenyl acetate could be acetylated to yield the tetraacetate **7**, $C_{22}H_{24}Cl_2O_{10}$. Thus, the second chlorine atom had been introduced at C-6 to give a C-1 hydroxyl group.

Treatment of crotepoxide (**1**) with methanolic hydriodic acid yielded two products. The less polar material was the iodohydrin **8**, $C_{18}H_{19}IO_8$, and the more polar the ene diol **9**, $C_{18}H_{20}O_8$. The nmr spectrum



of **8** was very similar to that of **4** and the coupling constants of the C-2, C-3, C-4, and C-5 protons (Table II) suggested that the protons were all approximately mutually antiperiplanar. Thus, it may be deduced

that, if the opening of the 4,5-epoxide had proceeded in a *trans*-diaxial fashion, then crotepoxide (**1**) has the relative stereochemistry of C-2 to C-5 as shown. The confirmation of this conclusion and the assignment of an absolute stereochemistry to **1** were obtained from an X-ray crystallographic analysis[6] of **8**. In the crystal, the ring adopts a half-chair confirmation with neighboring hydroxyl and acetoxyl substituents oriented equatorially. In the nmr spectrum of **1**, the coupling constants are in agreement with the presence of diaxial protons at C-2 and C-3 and of a C-4 proton in the plane of the ring. It is interesting to compare this with the conformation of senepoxide, proposed by Polonsky and coworkers,[7] where the C-2 and C-3 acetates are diaxially situated. This less favorable conformation is apparently due to the eclipsing of the C-2 acetate and the C-7 methylene grouping in what would, at first sight, appear to be a more stable equatorial conformation, In the case of crotepoxide, due to a different ring shape and a different stereochemistry at C-1, the C-2 acetate and C-7 methylene function are not eclipsed when the acetates are equatorially situated. The ring can therefore favorably adopt the more stable conformation.

It is apparent that initial *trans*-diaxial opening of the epoxide would lead initially to an intermediate in an unfavorable boat conformation. That this is followed by inversion to the half-chair form with the neighboring iodine and hydroxyl group in equatorial conformations is clearly shown from the X-ray analysis and nmr data discussed above.[8]

The nmr spectrum of the ene diol **9** contained two D_2O-exchangeable proton signals at τ 6.5–6.8 and a broad singlet at τ 4.10 (2 H) for the vinylic protons. The ene diol **9** was converted under mild acylating conditions to the triacetate **10**; with prolonged treatment an oil was obtained which was tentatively assigned the structure **11** on the basis of its spectral properties.

Oxidation of **9** with 8 *N* chromic acid gave an oil, whose properties were in agreement with structure **13**. The nmr spectrum contained signals at τ 3.05 and 3.76 (J = 11.0 Hz), corresponding to an α,β-unsaturated ketone. There was enhanced absorption at 227 mμ in the ultraviolet spectrum and a band at 6.03 μ in the infrared spectrum, both also attributable to an α,β-unsaturated ketone. Hydrogenation of **13** gave the saturated ketone **12**, $C_{18}H_{20}O_8$. The nmr spectrum of **12** lacked vinylic proton signals but contained a multiplet between τ 6.8 and 8.2 (4 H) assignable to the C-5 and C-6 methylene protons.

It was deemed desirable to convert crotepoxide to an aromatic derivative and thus confirm chemically the presence of the cyclohexane ring. Hence, the ene diol **9** was treated with thionyl chloride to give a dichloro compound, $C_{18}H_{18}Cl_2O_6$. However, it was not possible to assign the chlorine atom positions. Dehydrohalogenation of the dichloro compound with zinc powder yielded a number of products, but it was not possible to isolate an aromatized derivative.

(6) S. M. Kupchan, R. J. Hemingway, P. Coggon, A. T. McPhail, and G. A. Sim, *J. Amer. Chem. Soc.*, **90**, 2982 (1968); P. Coggon, A. T. McPhail, and G. A. Sim, *J. Chem. Soc., B*, 534 (1969).

(7) R. Hollands, D. Becher, A. Gaudemer, J. Polonsky, and N. Ricroch, *Tetrahedron*, **24**, 1633 (1968).

(8) *Cf.* D. H. R. Barton, D. A. Lewis, and J. F. McGhie, *J. Chem. Soc.*, 2907 (1957).

Crotepoxide belongs to a small group of naturally occurring, highly oxygenated cyclohexane derivatives other members of which are terreic acid,[9] epoxydone,[10] senepoxide and seneol,[7] and shikimic acid. However, crotepoxide is the only member of this group which possesses the diepoxide functionality.[11] This function has been shown earlier to confer tumor-inhibitory activity on certain series of synthetic compounds.[12] Consequently, it appears likely that the diepoxide functionality is a highly significant feature among the complex of factors which impart tumor-inhibitory activity to crotepoxide.

Experimental Section

Melting points were determined on a Thomas–Hoover capillary melting point apparatus and are corrected. Values of $[\alpha]_D$ have been approximated to the nearest degree. Ultraviolet absorption spectra were determined on a Beckman Model DK-2A recording spectrophotometer. Infrared absorption spectra were determined on a Beckman Model 5A recording spectrophotometer. Nuclear magnetic resonance spectra were determined on a Varian A-60A spectrometer using tetramethylsilane as internal standard. Petroleum ether refers to the fraction with bp 60–68°. Evaporations were carried out below 40° under reduced pressure. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich.

Isolation of Crotepoxide (1).—The dried ground fruits of *C. macrostachys* (1.5 kg) were twice extracted continuously with 95% ethanol for 8 hr and the combined extracts were concentrated to a thick oil (A, 344 g). A portion of this extract (30 g) was partitioned between 10% aqueous methanol (150 ml) and petroleum ether (two 100-ml portions). The extracts were evaporated to give C (23.7 g) and B (3.5 g), respectively. A portion of ethanol-soluble fraction C (15 g) was partitioned between water (50 ml) and *n*-butanol (three 50-ml portions) and the combined butanol extracts were washed with water (20 ml). The butanol and aqueous extracts were evaporated to yield fractions D (10.0 g) and E (4.7 g), respectively. Fraction D was chromatographed on silicic acid (400 g). Elution with chloroform and then with 1% methanol in chloroform gave an active fraction F (1.4 g). Fraction F was rechromatographed on silicic acid (150 g) and, with 0.5% methanol in chloroform, a fraction (740 mg) was obtained which was crystallized from methanol to yield crude crotepoxide (590 mg). Recrystallization from methanol gave needles of **1** (355 mg): mp 150–151°; $[\alpha]^{25}D$ +74° (c 1.70, $CHCl_3$); uv λ_{max}^{MeOH} 274 (ϵ 1050) and 281 mμ (ϵ 860); ir $\lambda_{max}^{CHCl_3}$ 5.71, 5.78, 6.24, 7.87, 8.20 and 9.00 μ.

Anal. Calcd for $C_{18}H_{18}O_8$: C, 59.66; H, 5.01. Found: C, 59.84; H, 5.00.

Hydrolysis of Crotepoxide. A. Vigorous Conditions.—A solution of crotepoxide (50 mg) in 0.1 *N* aqueous sodium hydroxide (10 ml) was heated on a steam bath for 90 min. The solution was acidified and extracted with ether (30 ml). The ether extract was washed with water and dried (Na_2SO_4), and the ether was evaporated to leave a residue smelling of acetic acid. The acetic acid was removed *in vacuo* and the residual solid was crystallized from ether–petroleum ether to give colorless needles of benzoic acid, mp 121–122°.

B. Mild Conditions.—A solution of crotepoxide (305 mg) in chloroform (5 ml) was treated with a solution of potassium hydroxide (200 mg) in 20% aqueous methanol (10 ml). After 5 min, the solution was acidified to pH 5 with 1 *N* hydrochloric acid and the solvent was evaporated. The residue was extracted with 25% methanol in chloroform (5 ml) and the extract was diluted with chloroform (20 ml) and chromatographed on silicic acid (20 g) using methanol–chloroform mixtured as solvent. The main fraction (74 mg) crystallized from methanol–ether to give colorless needles of triol **2**: mp 101–102°; $[\alpha]^{27}D$ +30° (c 1.06, MeOH); ir λ_{max}^{Nujol} 3.10 and 7.12 μ.

(9) J. C. Sheehan, W. B. Lawson, and R. J. Gaul, *J. Amer. Chem. Soc.*, **80**, 5536 (1958).

(10) A. Closse, R. Mauli, and H. P. Sigg, *Helv. Chim. Acta*, **49**, 204 (1966).

(11) Crotepoxide has recently been isolated from *Piper futokadzura* [S. Takahashi, *Phytochemistry*, **8**, 321 (1969)].

(12) *Cf., e.g.*, J. L. Everett and G. A. R. Kon, *J. Chem. Soc.*, 3131 (1950).

Anal. Calcd for $C_7H_{10}O_5$: C, 48.27; H, 5.79. Found: C, 48.50; H, 5.91.

Hexahydrocrotepoxide (3).—A solution of crotepoxide (100 mg) in methanol (10 ml) was hydrogenated for 3 hr using platinum oxide (35 mg) as catalyst until 3 mol equiv of hydrogen was absorbed. The catalyst was removed by filtration and the solvent was evaporated to yield a white solid, which was crystallized from ether–petroleum ether to give colorless needles (65 mg) of hexahydrocrotepoxide (**3**): mp 121–122°; $[\alpha]^{30}D$ +59° (*c* 1.35, $CHCl_3$); ir λ_{max}^{Nujol} 5.73, 5.78, 5.82, 8.01, 8.17, 8.60, 11.11, and 11.48 μ.

Anal. Calcd for $C_{18}H_{24}O_8$: C, 58.69; H, 6.57. Found: C, 58.63; H, 6.48.

Crotepoxide Monochlorohydrin (4).—A solution of crotepoxide (240 mg) in chloroform (2 ml) was treated with 20% aqueous methanolic 2 *N* hydrochloric acid (10 ml) for 30 min at room temperature. The solvent was evaporated and the residue was dissolved in chloroform and chromatographed on silicic acid (20 g) using chloroform and 1% methanol in chloroform as solvents. The main fraction (164 mg) was crystallized from chloroform–benzene to give colorless needles of monochlorohydrin **4**: mp 170–171°; $[\alpha]^{29}D$ −4° (*c* 1.35, $CHCl_3$); uv λ_{max}^{MeOH} 274 (ϵ 1020) and 281 mμ (ϵ 840); ir $\lambda_{max}^{CHCl_3}$ 2.90, 5.73, 5.85, 6.22, 6.32, 7.97, 8.08, 14.00, and 14.55 μ.

Anal. Calcd for $C_{18}H_{19}ClO_8$: C, 54.21; H, 4.70; Cl, 8.90. Found: C, 54.31; H, 4.80; Cl, 8.90.

Dideacetylcrotepoxide Dichlorohydrin (5).—A solution of crotepoxide (200 mg) in chloroform (2 ml) was treated with 20% aqueous methanolic 2 *N* hydrochloric acid (10 ml) at 40° for 16 hr. The solvent was evaporated and the residual glassy solid was extracted with hot chloroform (20 ml). The insoluble residue was crystallized from methanol to give colorless needles of dideacetylcrotepoxide dichlorohydrin (**5**): mp 241–242°; $[\alpha]^{25}D$ −10° (*c* 0.71, MeOH); uv λ_{max}^{MeOH} 274 (ϵ 1000) and 281 mμ (ϵ 830); ir $\lambda_{max}^{CHCl_3}$ 3.00, 5.88, 6.25, 7.85, 14.10, and 14.55 μ.

Anal. Calcd for $C_{14}H_{16}Cl_2O_6$: C, 47.88; H, 4.59; Cl, 20.19. Found: C, 48.09; H, 4.50; Cl, 20.20.

Triacetate of Dideacetylcrotepoxide Dichlorohydrin (6).—A solution of dideacetylcrotepoxide dichlorohydrin (**5**, 60 mg) in pyridine (1 ml) was treated with acetic anhydride (100 mg) and the mixture was allowed to stand at room temperature for 16 hr. The solvent was evaporated and the residue was chromatographed on silicic acid (6 g) using chloroform as solvent. The first fraction (85 mg) crystallized from chloroform–hexane to give colorless needles of the triacetate **6**: mp 217–218°; $[\alpha]^{25}D$ −34° (*c* 1.18, $CHCl_3$); uv λ_{max}^{MeOH} 230 (ϵ 10,500), 274 (ϵ 960), and 281 mμ (ϵ 790); ir $\lambda_{max}^{CHCl_3}$ 3.00, 3.30, 5.69, 7.27, and 8.00 μ.

Anal. Calcd for $C_{20}H_{22}Cl_2O_9$: C, 50.32; H, 4.65; Cl, 14.85. Found: C, 50.38; H, 4.68; Cl, 14.94.

Tetraacetate of Dideacetylcrotepoxide Dichlorohydrin (7).—A solution of dideacetylcrotepoxide dichlorohydrin (50 mg) and *p*-toluenesulfonic acid (2 mg) in isopropenyl acetate (3 ml) was heated to 60–70° for 16 hr. The solvent was evaporated and the residue was chromatographed on silica gel (10 g) using chloroform as solvent. The first fraction (60 mg) was crystallized from ether–hexane to give colorless prisms of the tetraacetate **7**: mp 153–154°; $[\alpha]^{27}D$ −61° (*c* 0.91, $CHCl_3$); uv λ_{max}^{MeOH} 230 (ϵ 13,600), 274 (ϵ 1030), and 281 mμ (ϵ 835); ir $\lambda_{max}^{CHCl_3}$ 3.30, 5.70, 5.75, 6.23, 6.87, 7.30, 7.85, 8.10, 9.30, and 9.56 μ.

Anal. Calcd for $C_{22}H_{24}Cl_2O_{10}$: C, 54.21; H, 4.96; Cl, 14.55. Found: C, 54.06; H, 5.02; Cl, 14.61.

Crotepoxide Monoiodohydrin (8).—A solution of crotepoxide (450 mg) in chloroform (10 ml) was treated with a solution of 10% aqueous methanolic hydriodic acid (10 ml) prepared from iodine (2 g) and an excess of hydrogen sulfide, the precipitated sulfur being removed by filtration. After 1 hr at room temperature, a saturated aqueous solution of sodium thiosulfate (2 ml) was added and the solution was evaporated to a small volume. The concentrated solution was diluted with water (5 ml) and extracted with chloroform. The chloroform solution was dried (Na_2SO_4) and evaporated. The residue was dissolved in chloroform and chromatographed on silicic acid (25 g). The fraction (130 mg), eluted with 1% methanol in chloroform, was crystallized from ether to give colorless plates of crotepoxide monoiodohydrin (**8**): mp 143–144°; $[\alpha]^{28}D$ −46° (*c* 1.40, $CHCl_3$); uv λ_{max}^{MeOH} 274 (ϵ 1420) and 281 mμ (ϵ 1130); ir $\lambda_{max}^{CHCl_3}$ 2.90, 5.78, 5.85, 6.23, 6.30, 8.10, 14.00, and 14.50 μ.

Anal. Calcd for $C_{18}H_{19}IO_8$: C, 43.94; H, 3.89; I, 25.79. Found: C, 43.92; H, 3.89; I, 25.91.

Ene Diol Derivative (9) of Crotepoxide.—From the preparation of crotepoxide monoiodohydrin, a fraction (255 mg) eluted with 3% methanol in chloroform was crystallized from methanol–ether to give colorless needles of crotepoxide ene diol (**9**): mp 145–146°; $[\alpha]^{28}D$ +127° (*c* 1.51, $CHCl_3$); uv λ_{max}^{MeOH} 274 (ϵ 970) and 281 mμ (ϵ 790); ir $\lambda_{max}^{CHCl_3}$ 3.00, 5.70, 5.82, 6.27, 7.87, 8.12, and 14.07 μ.

Anal. Calcd for $C_{18}H_{20}O_8$: C, 59.33; H, 5.33. Found: C, 59.25; H, 5.51.

Triacetate 10.—A solution of ene diol **9** (60 mg) in pyridine (2 ml) was treated with acetic anhydride (0.5 ml) and allowed to stand at room temperature for 12 hr. The solvent was evaporated and the residue was dissolved in chloroform and chromatographed on silicic acid (10 g). The main fraction (55 mg) was crystallized from benzene–hexane to give colorless prisms of triacetate **10**: mp 141–142°; $[\alpha]^{28}D$ +151° (*c* 0.91, $CHCl_3$); uv λ_{max}^{MeOH} 274 (ϵ 960) and 281 mμ (ϵ 780); ir $\lambda_{max}^{CHCl_3}$ 2.83, 3.30, 5.75, 6.25, 6.90, 7.30, 7.88, 8.10, and 14.13 μ.

Tetraacetate 11.—A solution of ene diol **9** (60 mg) in acetic anhydride (2 ml) was refluxed for 5 hr. The solvent was evaporated and the residue was dissolved in 50% benzene–chloroform and chromatographed on silicic acid (8 g). The main fraction (48 mg) was a colorless oil **11**, which could not be crystallized: ir λ_{max}^{film} 3.42, 5.74, 6.28, 6.91, 7.34, 7.87, 8.15, and 9.00 μ.

Oxidation of Ene Diol 9.—A solution of ene diol **9** (80 mg) in acetone (4 ml) was treated with 8 *N* chromic acid (1.0 ml) at 0° for 6 min. The product was diluted with water (15 ml) and extracted with chloroform (50 ml). The chloroform extracts were dried (Na_2SO_4) and evaporated to yield an oil (80 mg). The product was chromatographed on silicic acid (10 g) using chloroform as solvent, to give an oil (**13**, 72 mg), which could not be crystallized: uv λ_{max}^{MeOH} 227 (ϵ 19,100), 273 (ϵ 2200), and 280 mμ (ϵ 880); ir λ_{max}^{film} 2.93, 3.30, 5.70, 5.80, 6.03, 6.25, 6.90, 7.30, 7.88, and 8.15 μ.

Hydrogenation of α,β-Unsaturated Ketone 13.—A solution of **13** (60 mg) in methanol (5 ml) was hydrogenated for 1 hr at room temperature, with 10% Pd–C (25 mg) as catalyst. The catalyst was removed by filtration and the solvent was evaporated to give an oil (60 mg). Crystallization from ether–hexane gave colorless needles of the saturated ketone **12**: mp 115–116°; $[\alpha]^{24}D$ −3° (*c* 1.00, $CHCl_3$); uv λ_{max}^{MeOH} 229 (ϵ 11,200), 274 (ϵ 960), and 281 mμ (ϵ 780); ir $\lambda_{max}^{CHCl_3}$ 2.80, 5.69, 5.74, 6.23, 7.26, 7.85, 8.10, 9.00, and 9.55 μ.

Anal. Calcd for $C_{18}H_{20}O_8$: C, 59.33; H, 5.33. Found: C, 59.36; H, 5.39.

Thionyl Chloride Treatment of Ene Diol 9.—A solution of ene diol **9** (250 mg) in pyridine (0.5 ml) was treated with a solution of thionyl chloride (200 mg) in pyridine (1.5 ml). After 10 min at room temperature, chloroform (20 ml) was added, the solution was cooled in an ice–salt bath, and ice–water was added. The mixture was acidified, the chloroform was separated, and the aqueous layer was extracted with chloroform (50 ml). The combined chloroform extracts were dried (Na_2SO_4) and evaporated. The residue was chromatographed on silicic acid (20 g). The main fraction (180 mg) was crystallized from ether to yield colorless needles of a dichloro compound: mp 182–184°; $[\alpha]^{28}D$ +41° (*c* 0.59, $CHCl_3$); uv λ_{max}^{MeOH} 274 (ϵ 1000) and 281 mμ (ϵ 815); ir $\lambda_{max}^{CHCl_3}$ 5.72, 5.81, 6.25, 6.90, 7.30, 7.90, 8.10, and 14.15 μ.

Anal. Calcd for $C_{18}H_{18}Cl_2O_8$: C, 53.92; H, 4.52; Cl, 17.68. Found: C, 53.86; H, 4.41; Cl, 17.85.

Dehydrohalogenation of the Dichloro Compound.—A solution of the dichloro compound (34 mg) in methanol (1 ml) was treated with acetic acid (0.2 ml) and zinc powder (50 mg) and the mixture was warmed to 60° for 30 min. After a further 3 hr at room temperature, the zinc was removed by filtration, the filtrate was evaporated, and the residue was chromatographed on silicic acid (7 g). The major fraction (17 mg), eluted with chloroform, was an oily diene which could not be crystallized: uv λ_{max}^{MeOH} 264 mμ (ϵ 6600).

Registry No.—**1**, 21887-30-9; **2**, 20421-15-2; **3**, 21887-32-1; **4**, 20421-16-3; **5**, 20421-17-4; **6**, 20421-18-5; **7**, 20421-19-6; **8**, 20421-20-9; **9**, 20421-21-0; **10**, 21887-39-8; **11**, 21887-40-1; **12**, 20421-23-2; **13**, 21887-42-3.