doses to produce a 50% increase in life span.

As a first step toward determining the possible metabolic fate of these compounds in vivo, the extent of demethylation of compound 1 by liver microsomes from phenobarbitone-pretreated CBA/LAC mice was investigated by the method of Cochin and Axelrod.[8] As reported previously, no detectable formaldehyde was formed under these conditions.[9] The reason for this apparent lack of metabolism in vitro is currently the subject of further investigation.

Experimental Section

Melting points were determined on a Reichert, Kofler micro hot stage apparatus and are uncorrected. Ultraviolet spectra were recorded in ethanol solution on a Pye SP800 spectrometer. Elemental analyses were obtained from Butterworth Laboratories Ltd., Teddington, England, or from Dr. F. B. Strauss, Oxford, England.

Triazene Synthesis. All the amines used were obtained commercially and used without further purification, with the exception of *N*-methylpentylamine which was prepared by the method of Lucier and co-workers.[10] The physicochemical properties of the new compounds are described in Table I. The ultraviolet absorbance in the region of 323 nm is characteristic of the triazene structure. The preparation of compound **5** is typical.

1-(4-Carboxyphenyl)-3-methyl-3-pentyltriazene (5). A solution of 5.5 g (0.04 mol) of 4-aminobenzoic acid in 100 mL (0.1 mol) of hydrochloric acid was diazotized at 0 °C by the addition of 2.76 g (0.04 mol) of sodium nitrite in 10 mL of water. After 0.5 h, excess nitrite was decomposed with sulfamic acid, and a mixture of 5.47 g (0.04 mol) of *N*-methylpentylamine hydrochloride and 6.0 g (0.15 mol) of sodium hydroxide in 25 mL of water was added. When the reaction was complete (no coloration of alkaline α-naphthol solution), 50% aqueous acetic acid was added to reduce the pH to 4–5. The mixture was extracted with ethyl acetate, the organic phase was dried over Na_2SO_4, and the solvent was removed under reduced pressure. Crystallization of the residue from petroleum ether (bp 60–80 °C), followed by recrystallization from cyclohexane, yielded 6.3 g (63%), mp 132–134 °C. Anal. ($C_{13}H_{19}N_3O_2$) C, H, N.

Solubility Determination. Solubilities were determined by shaking an excess of the compound in 0.1 M Sorenson's pH 7.5 phosphate buffer in a water bath at 25 °C for 1 h. The mixture was rapidly filtered, and an aliquot was diluted to a known volume with ethanol. The resulting solution was passed through a Millex 25 (Millipore) disposable filter and the absorbance measured at the λ_{max}. The solubility was then calculated from the standard spectral data.

Partition Coefficient Determination. The compound was shaken at room temperature for 1 h in 0.1 M Sorenson's pH 7.5 phosphate buffer previously saturated with octanol. A known volume of this filtered solution was shaken with a known volume of buffer saturated octanol for 1 h at room temperature. Aliquots of the aqueous phase and the initial filtrate were independently treated in the same manner as the filtrate in the solubility determination. The partition coefficient, *P*, was calculated from the expression:

$$P = \text{vol of aqueous phase} \times (\text{initial absorbance} - \text{final absorbance}) / \text{vol of octanol phase} \times \text{final absorbance of aqueous phase}$$

The results in Table I are the mean of at least two determinations.

Antitumor Activity. The TLX/5 lymphoma was transplanted subcutaneously in female CBA/LAC mice as previously described.[9] The triazenes were administered intraperitoneally in saline (Table II, footnote *a*) for 5 consecutive days, commencing 3 days after tumor transplantation. Groups of five animals were used for each dose level, and their survival time was compared with ten untreated control mice. An increase in life span of 20% or greater is statistically significant.

Acknowledgment. This work was supported by grants to the Institute of Cancer Research: Royal Cancer Hospital, from the Cancer Research Campaign and the Medical Research Council. We are grateful to Professor W. C. J. Ross and Dr. K. R. Harrap for their interest.

(8) J. Cochin and J. Axelrod, *J. Pharmacol. Exp. Ther.*, **125**, 105 (1959).
(9) R. C. S. Audette, T. A. Connors, H. G. Mandel, K. Merai, and W. C. J. Ross, *Biochem. Pharmacol.*, **22**, 1855 (1973).
(10) J. J. Lucier, A. D. Harris, and P. S. Korosec, "Organic Syntheses", Collect. Vol. V, Wiley, New York, 1973, p 736.

Antileukemic Compounds Derived from the Chemical Modification of Macrocyclic Trichothecenes. 1. Derivatives of Verrucarin A

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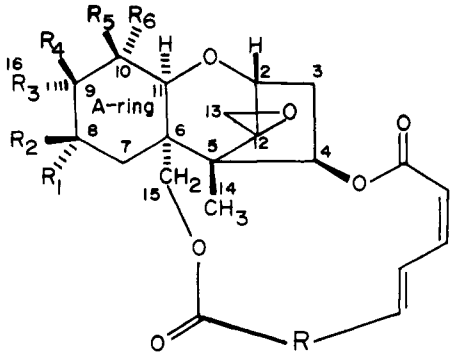
Verrucarin A (**2**) was epoxidized to give the β-9,10-epoxide **7** (major product) and α-9,10-epoxide **9** (minor product). The β-epoxide **7** and its acetate **8** exhibit high in vivo antileukemic activity against P-388 mouse leukemia, whereas **2** and **9** are inactive. Epoxidation of verrucarin B (**3**) and roridin A (**1**) to their respective β-9,10-epoxides (**11** and **12**, respectively) also yields compounds with substantially increased activity. Allylic alcohols derived from **2**, α-C8 (**20**), β-C8 (**14**), and C16 (**15**), were synthesized and tested; only **15** exhibited substantial in vivo activity.

The search for biologically active compounds from the extracts of higher plants, microbial fermentations, and marine animals has yielded a vast array of new and interesting natural products. Through a random search of a large number of these extracts, a significant number of materials have been isolated and identified which show potential for use as antineoplastic agents.[1] Some of these have served as useful prototypes for further chemical modification which potentially could lead to more effective drugs.[1]

(1) (a) Douros, J.; Suffness, M. *Cancer Chemother. Pharmacol.* **1978**, *1*, 91. (b) Suffness, J.; Douros, J. *Methods Cancer Res.* **1979**, *16*, 73.

0022-2623/80/1823-1054$01.00/0

Chart I



COMPOUND	R	R_1	R_2	R_3	R_4	R_5	R_6
Roridin A (1)	$-CH(OH)CH(CH_3)CH_2CH_2OCH(CHOHCH_3)-$	H	H	CH_3	–	–	H
Verrucarin A (2)	$-CH(OH)CH(CH_3)CH_2CH_2OC(=O)-$	H	H	CH_3	–	–	H
Verrucarin B (3)	$-CH(O)C(CH_3)CH_2CH_2O-C(=O)-$	H	H	CH_3	–	–	H
Baccharin (4)	$-CH(O)C(CH_3)CH(OH)CH_2OCH(CHOHCH_3)-$	H	H	CH_3	O		H
Baccharinol (5)	$-CH(O)C(CH_3)CH(OH)CH_2OCH(CHOHCH_3)-$	H	OH	CH_3	–	–	H
Baccharinol (6)	$-CH(OH)CH(CH_3)CH_2CH_2OCH(CHOHCH_3)-$	H	OH	CH_3	–	–	H
9β,10β-Epoxyverrucarin A (7)	$-CH(OH)CH(CH_3)CH_2CH_2OC(=O)-$	H	H	CH_3	O		H
9β,10β-Epoxyverrucarin A Acetate (8)	$-CH(OAc)CH(CH_3)CH_2CH_2OC(=O)-$	H	H	CH_3	O		H
9α,10α-Epoxyverrucarin A (9)	$-CH(OH)CH(CH_3)CH_2CH_2OC(=O)-$	H	H	a	CH_3	H	a
9β-Hydroxy-10α-bromoverrucarin A (10)	$-CH(OH)CH(CH_3)CH_2CH_2OC(=O)-$	H	H	CH_3	OH	H	Br
9β,10β-Epoxyverrucarin B (11)	$-CH(O)C(CH_3)CH_2CH_2OC(=O)-$	H	H	CH_3	O		H
9β,10β-Epoxyroridin A (12)	$-CH(OH)CH(CH_3)CH_2CH_2OCH(CHOHCH_3)-$	H	H	CH_3	O		H
9β,10β-Epoxyroridin A Diacetate (13)	$-CH(OAc)CH(CH_3)CH_2CH_2OCH(CHOAcCH_3)-$	H	H	CH_3	O		H
8β-Hydroxyverrucarin A (14)	$-CH(OH)CH(CH_3)CH_2CH_2OC(=O)-$	H	OH	CH_3	–	–	H
16-Hydroxyverrucarin A (15)	$-CH(OH)CH(CH_3)CH_2CH_2OC(=O)-$	H	H	CH_2OH	–	–	H
8-Oxoverrucarin A (17)	$-CH(OH)CH(CH_3)CH_2CH_2OC(=O)-$	O		CH_3	–	–	H
16-Oxoverrucarin A (18)	$-CH(OH)CH(CH_3)CH_2CH_2OC(=O)-$	H	H	CHO	–	–	H
8α-Hydroxy-9,10-dihydroverrucarin A (19)	$-CH(OH)CH(CH_3)CH_2CH_2OC(=O)-$	OH	H	CH_3,H[b]		H	H
8α-Hydroxyverrucarin A (20)	$-CH(OH)CH(CH_3)CH_2CH_2OC(=O)-$	OH	H	CH_3	–	–	H

[a] R_3, R_6 = epoxy
[b] Stereochemistry at C_9 unassigned

Several years ago,[2] such a search showed that an extract of *Baccharis megapotamica*, a shrub found in Brazil, exhibited very high in vivo activity against P-388 mouse leukemia. Fractionation of this extract led to the isolation of a series of antileukemic macrocyclic sesquiterpenes known as baccharins and baccharols which belong to a class of compounds known as trichothecenes.[3]

From the standpoint of biological activity, the baccharins represent the most in vivo P-388 active macrocyclic trichothecenes isolated.[1a] A comparison of their structures with those of the in vivo inactive macrocyclic trichothecenes [e.g., roridin A (1) and verrucarin A (2)] suggested

(2) (a) Kupchan, S. M.; Jarvis, B. B.; Dailey, R. G., Jr.; Bright, W.; Bryan, Y. F.; Shizuri, Y. *J. Am. Chem. Soc.* **1976**, *98*, 7092. (b) Kupchan, S. M.; Streelman, D. R.; Jarvis, B. B.; Dailey, R. G., Jr.; Sneden, A. T. *J. Org. Chem.* **1977**, *42*, 4221.

(3) For a review, see Tamm, C. *Fortscher. Chem. Org. Naturst.* **1974**, *31*, 63.

Table I. In Vivo P-388 Mouse Leukemia (PS Activity) of the Macrocyclic Trichothecenes

compound	P-388 dose, mg/kg[a]	T/C
verrucarin A (2)	2	127
verrucarin A acetate	>1	toxic
verrucarin B (3)	5	142
verrucarin J[b]	0.8	150
roridan A (1)	0.06	128
roridin A (1)	≤2	<125
roridin D[b]	12.5	131
roridin J[c]	5	158
baccharin B1[d]	1.25	214
baccharin B2[d]	2.5	278
baccharin B3 (6)	5.0	250
baccharin B4 (5)	2.5	185
baccharin B5 (4)	5.0	311
baccharin B6 (5)	2.5	166
baccharin B7 (6)	0.62	196
baccharin B8 (4)	2.5	233
β-9,10-epoxyverrucarin A (7)	8	210
β-9,10-epoxyverrucarin A acetate (8)	5	172
α-9,10-epoxyverrucarin A (9)	2.5	118
β-8-hydroxyverrucarin A (14)	1.25	132
α-8-hydroxyverrucarin A (20)	≥1	toxic
8-oxoverrucarin A (17)	12.5	122
16-hydroxyverrucarin A (15)	1	190
β-9-hydroxy-α10-bromoverrucarin A (10)	10	133
β-9,10-epoxyverrucarin B (11)	10	157
β-9,10-epoxyroridin A (12)	10	205

[a] The dose levels are those which exhibit the highest T/C values. Dose levels higher are generally toxic. [b] See ref 4 for structure. [c] Jarvis, B. B.; Stahly, G. P.; Pavanasasivam, G; Mazzola, E. P *J. Antibiot.* **1980**, *33*, 256. [d] The structure of B1 and B2 are similar to B3–B7, except that the hydroxyl group at C2′ is located at C4′; see ref 2b for details concerning the structures of these compounds.

that chemical modification of the inactive mycotoxins would lead to compounds with substantially improved in vivo activity.

Results and Discussion

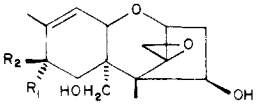
The structures of a number of the macrocyclic trichothecenes are shown in Chart I. Although the structural differences between **1–3** and **4–6** appear slight, the in vivo P-338 activity differences are very large (Table I). The roridins (A, D, and H) and verrucarins (A, B, and J) exhibit T/C values <160, whereas the baccharins exhibit T/C values >160.[4,5] The key structural difference between the highly active baccharins and the considerably less active roridins and verrucarins appears to be the presence of an oxygen substituent in the A ring of the baccharins.

It seems to make little difference in P-388 activity whether the baccharins possess a C8 allylic hydroxyl group or a C9, C10 epoxy group, as both **4** and **5** show similar activity. The dependence of activity on the stereochemistry of these oxygen atoms is not known, however, since only the β isomers of **4**, **5**, and related compounds have been isolated. We have oxidized several verrucarins and roridins to determine if the introduction of oxygen atoms into the A rings of these compounds will impart in vivo activity to them. In addition, we have varied the position and stereochemistry of the oxygen atoms to determine what effect such factors have on in vivo activity.

Oxidation of verrucarin A (**2**)[6] with *m*-chloroperbenzoic acid (MCPBA) in chloroform[7] gave the 9β,10β-epoxide **7** (66%), along with a minor amount of the 9α,10α-epoxide **9** (6%).[8] The proof of structure for **7** and **9** is based on precedent[10] as well as NMR spectral data. In particular, $J_{10,11}$ for epoxides **7** and **9** differ significantly in that $J_{cis\text{-}10,11}$ = 4 Hz (**7**), whereas $J_{trans\text{-}10,11}$ = 2 Hz (**9**). These coupling constants are typical for substituted epoxides. Furthermore, earlier workers[10a] epoxidized verrucarin A acetate to give predominately **8**, which proved identical with the compound we obtained on acetylation of **7**.

We also epoxidized verrucarin B (**3**) and roridin A (**1**) with MCPBA in chloroform. Both of these yielded β-epoxides stereoselectively and, due to the small scale on which these preparative reactions were carried out, only the major products were isolated; 9β,10β-epoxyverrucarin B (**11**) was isolated in 57% yield, while 9β,10β-epoxyroridin A (**12**) was isolated in 63% yield. Minor products, presumed to be α-epoxides from their R_f values on silica gel, were observed on TLC analyses of the reaction mixtures. The stereochemical assignments of the β-epoxides **11** and **12** also were based on the $J_{10,11}$ values observed in the proton NMR spectra, and **12** was acetylated to give the known β-epoxyroridin A diacetate **13**.[10b]

Allylic oxidation of verrucarin A (**2**) was carried out to generate a compound resembling baccharinol. Selenium dioxide, the reagent of choice for allylic hydroxylation,[11] was found to react with **2** at 100 °C in acetic acid and acetic anhydride to produce a mixture of alcohols **14** (48%) and **15** (8%), which were identified by their NMR spectra.[12] The β configuration at C8 of compound **14** was determined by hydrolysis of the macrocycle and recovery of trihydroxytrichothecene **16**. Triol **16** proved identical,



16, R_1 = H, R_2 = OH
21, R_1 = OH, R_2 = H

physically and spectrally, with the known compound obtained from methanolysis of baccharinol.[2b] The C8 configuration of baccharinol has been established by an X-ray crystallographic study.[2b]

Synthesis of the α-epimeric allylic alcohol was accomplished via an oxidation–reduction procedure. Both **14** and

(4) Douros, J., private communication.
(5) T/C = (days test animals live/days control animals live) × 100. A T/C value >125 is considered significant.
(6) Verrucarins A and B and roridin A were isolated from fermentations of *Myrothecium verrucaria* (ATCC no. 24571). Details of this procedure will be presented in a forthcoming publication.
(7) A preliminary account of this work has appeared: Jarvis, B. B.; Stahly, G. P.; Curtis, C. R. *Cancer Treat. Rep.* **1978**, *62*, 1585.
(8) Attempts at obtaining higher yields of the α-epoxide by use of various oxidizing agents were unsuccessful. The classical method[9] of forming the bromohydrin gave only compound **10**, which upon dehydrobromination gave only the β-epoxide **7**.
(9) Berti, G. *Top. Stereochem.* **1973**, *7*, 187.
(10) (a) Zurcher, W.; Gutzwiller, J.; Tamm, C. *Helv. Chim. Acta* **1965**, *48*, 840. (b) Achini, R.; Tamm, C. *Ibid.* **1968**, *51*, 1712.
(11) Fieser, L. F.; Fieser, M. "Reagents for Organic Synthesis"; Wiley: New York, 1967; Vol. 1, p 994.
(12) See paragraph at the end of paper concerning supplementary material.

15 were oxidized with Collins reagent[13] to afford the unsaturated ketone 17 (82%) and aldehyde 18 (63%), respectively. A similar conversion of baccharinol has been carried out using pyridinium chlorochromate.[2b] Treatment of 17 with sodium borohydride at 0 °C in a mixture of THF and ethanol yielded two alcohols; formed selectively was the saturated alcohol 19 (56%), while the minor product proved to be the desired α-alcohol 20 (36%). Reduction of 17 at −35 °C gave 62% yield of the desired α-alcohol 20 accompanied by only 5% of the hydrogenated alcohol 19. Hydrolysis of 20 furnished triol 21.

The in vivo P-388 activities[14] of the naturally occurring and synthetically modified trichothecenes are shown in Table I. The conversions of verrucarin A and roridin A to their β-9,10-epoxides (7 and 12, respectively) result in compounds with a marked increase in their in vivo P-388 activity. Similar, though less marked, increases in P-388 activity are observed for verrucarin A acetate and verrucarin B (3) when they are converted to their respective β-9,10-epoxides. Thus, the original idea, based on a structure–activity relationship between the inactive roridins and verrucarins and the active baccharin β-epoxides, that epoxidation of the 9,10 double bond of the A ring in roridins and verrucarins would lead to marked increases in in vivo P-388 activity has been realized.[15] Furthermore, it appears that epoxidation must occur β in order to realize marked increases in in vivo activity, since the α-9,10-epoxide 9 is devoid of significant in vivo P-388 activity. The conclusions that can be drawn with respect to structure–activity relationships of the A ring hydroxylated macrocyclic trichothecenes are less clear. In the case of the baccharins, there does not appear to be a marked difference in the activity of the 9β,10β-epoxybaccharins and the 8β-hydroxybaccharins. However, the 8-hydroxyverrucarin A 14 and 20 show no significant increase in activity, although the β-epimer 14 is significantly less toxic than verrucarin A whereas the α-epimer 20 is more toxic. That the baccharins are derivatives of the roridins, whereas 14 and 20 are derived from verrucarins, may prove important relative to in vivo activity. Additional examples of these derivatives are being prepared and tested, and such data should shed further light on this point. It is interesting that the 16-hydroxy derivative 15 exhibits toxicity similar to verrucarin A and roridin A but also shows marked in vivo activity in the lower dose ranges.

Experimental Section

Melting points were determined on a Fisher-Johns hot stage melting point apparatus and are uncorrected. Infrared spectra were determined in chloroform or potassium bromide on a Perkin-Elmer 281 recording spectrophotometer. The polystyrene absorption at 1601.8 cm^{-1} was used for calibration of the infrared spectra. Nuclear magnetic resonance spectra were determined in deuteriochloroform or perdeuterated dimethyl sulfoxide on a Varian XL-100, EM-390, or FT-80 spectrometer with tetramethylsilane as an internal standard.[12] Microanalyses were carried out by Dr. Franz Kasler of the University of Maryland. Petroleum ether refers to the fraction of boiling point 35–60 °C. *p*-Dioxane was distilled from sodium and stored in the dark. Thin-layer chromatography was carried out on prepared silica gel plates (E. Merck or Analtech), and visualization was effected with short-wavelength UV light or sulfuric acid/ethanol/vanillin (20:3:1) spray. Flash chromatography was carried out on 230–400 mesh silica gel (E. Merck) as described by Still et al.[16]

Epoxidations of Verrucarins A and B and Roridin A. To a solution of 1.00 g (1.99 mmol) of verrucarin A (2) in 25 mL of chloroform was added 450 mg (2.20 mmol) of 85% *m*-chloroperbenzoic acid (MCPBA, Aldrich). After 24 h, the mixture was washed once with 25 mL of saturated sodium bicarbonate, dried ($MgSO_4$), and concentrated in vacuo. The residue was subjected to flash chromatography on 100 g of silica gel with 50% ethyl acetate in petroleum ether as eluent to give 676 mg (66%) of 9β,10β-epoxyverrucarin A (7) and 64.5 mg (6.3%) of 9α,10α-epoxyverrucarin A (9).

Recrystallization from dichloromethane–ether provided an analytical sample of 7: mp >300 °C; $[\alpha]^{18}_D$ +150° (*c* 1.90, $CHCl_3$); IR ($CHCl_3$) 3560, 1715, 1635, 1590 cm^{-1}. Anal. ($C_{27}H_{34}O_{10}$) C, H.

Recrystallization from dichloromethane–ether provided an analytical sample of 9: mp 250–252 °C; $[\alpha]^{26}_D$ +175° (*c* 3.07, $CHCl_3$); IR ($CHCl_3$) 3560, 1715, 1635, 1590 cm^{-1}. Anal. ($C_{27}H_{34}O_{10}\cdot 0.5H_2O$) C, H.

Acetylation of 9β,10β-epoxyverrucarin A (7) (acetic anhydride–pyridine) gave the known acetate 8[10a] in 72% yield.

Epoxidation of verrucarin B (3) and roridin A (1) in a like manner gave the β-epoxides 11 and 12, respectively. For 11: mp >300 °C; $[\alpha]^{26}_D$ +68.3° (*c* 2.49, $CHCl_3$); IR ($CHCl_3$) 1750, 1710, 1630, 1590 cm^{-1}. Anal. ($C_{27}H_{32}O_{10}$) C, H. For 12: mp 227–231 °C; $[\alpha]^{26}_D$ +101° (*c* 4.06, $CHCl_3$); IR ($CHCl_3$) 3560, 1725, 1640, 1600 cm^{-1}. Anal. ($C_{29}H_{38}O_{10}\cdot 0.5H_2O$) C, H.

Acetylation of 12 (acetic anhydride–pyridine) gave the known diacetate[10b] of roridin A in 62% yield.

9β-Hydroxy-10α-bromoverrucarin A (10). To a solution of 200 mg (0.40 mmol) of verrucarin A (2) in 75% aqueous *p*-dioxane was added 170 mg (1.4 mmol) of *N*-bromoacetamide.[17] After 90 min at room temperature, the mixture was poured into 25 mL of water and extracted with three 10-mL portions of dichloromethane. The combined organics were washed once with 25 mL of saturated sodium bisulfite, dried ($MgSO_4$), and concentrated in vacuo. Preparative TLC on silica gel (one 2-mm plate) with 40% petroleum ether in ethyl acetate as eluent yielded 162 mg (68%) of 10. Recrystallization from dichloromethane–ether provided an analytical sample of 10: mp 170–180 °C (with gas evolution); $[\alpha]^{29}_D$ +30.0° (*c* 1.61, $CHCl_3$); IR ($CHCl_3$) 3500, 1715, 1635, 1590 cm^{-1}. Anal. ($C_{27}H_3O_{10}Br$) C, H.

Treatment of the bromohydrin with Dowex 1-X4 basic ion-exchange resin in THF[18] gave 83% of β-epoxide 7.

Hydroxylation of Verrucarin A (2). A mixture of 1.85 g (0.369 mmol) of verrucarin A (2) and 2.05 g (1.85 mmol) of selenium dioxide in 15 mL each of glacial acetic acid and acetic anhydride was stirred in a 100 °C oil bath for 45 min. The reaction mixture was cooled, filtered, carefully poured into 250 mL of saturated sodium bicarbonate, and extracted with two 100-mL portions of dichloromethane. The combined organic layers were washed with two 200-mL portions of saturated sodium bicarbonate, dried ($MgSO_4$), and concentrated in vacuo. The residue was subjected to flash chromatography on 100 g of silica gel with 30% petroleum ether in ethyl acetate as eluent to give two fractions. The earlier eluted fraction provided 923 mg (48%) of 8β-hydroxyverrucarin A (14), an analytical sample of which was obtained by recrystallization from acetone–hexane: mp >300 °C; $[\alpha]^{25}_D$ +229° (*c* 1.37, CH_3OH); IR (KBr) 3500, 1710, 1630, 1590 cm^{-1}. Anal. ($C_{27}H_{34}O_{10}$) C, H.

Preparative TLC of the later eluted fraction on silica gel (one 2-mm plate) with ethyl acetate as eluent provided 155 mg (8.1%) of 16-hydroxyverrucarin A (15), an analytical sample of which was obtained by recrystallization from acetone–hexane: mp >300

(13) Collins, J. C.; Hess, W. W.; Frank, F. J. *Tetrahedron Lett.* **1968**, 3363.

(14) Antileukemic testing was carried out under the auspices of the NCI by the procedure described in Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*(2), 1–103.

(15) Although it is now evident that β-epoxidation of the 9,10 double bond in the macrocyclic trichothecenes leads to compounds with increased activity, this alteration does not appear to activate the simple nonmacrocyclic trichothecenes. Thus, epoxidation of the 9,10 double bond in 4β,15-diacetoxyscirpen-3α-ol (anquidine), a compound which possess high activity, gives a β-9,10-epoxide devoid of significant activity: Doyle, T. W., private communication.

(16) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

(17) Prepared as described in Oliveto, E. P.; Gerold, C. "Organic Syntheses"; Wiley: New York, 1963; Collect. Vol. IV, p 104.

°C; $[\alpha]^{25}_D$ +169° (c 1.65, CH_3OH); IR ($CHCl_3$) 3540, 1710, 1635, 1590 cm^{-1}. Anal. ($C_{27}H_{34}O_{10}$) C, H.

Hydrolysis of 8β-Hydroxyverrucarin A (14). A solution of 100 mg (0.19 mmol) of **14** and 81 mg (1.9 mmol) of lithium hydroxide monohydrate in 5 mL of methanol was stirred at room temperature for 3 h. The reaction mixture was concentrated in vacuo and the resulting residue was subjected to preparative TLC on silica gel (one 1-mm plate) with 20% methanol in dichloromethane as eluent to give 40 mg (74%) of 4β,8β,15-trihydroxy-12,13-epoxytrichothecene (**16**). Recrystallization from acetone–hexane provided an analytical sample of **16** which was identified by comparison (TLC behavior, mixture melting point, NMR spectra) with an authentic sample.[19] No effort was made to recover the other products of this reaction.

8-Oxoverrucarin A (17). To a mixture of 240 mg (2.4 mmol) of chromium trioxide and 1 mL of pyridine in 8 mL of dichloromethane was added 250 mg (0.48 mmol) of 8β-hydroxyverrucarin A (**14**). The reaction mixture was stirred for 1 h at room temperature, filtered, diluted to 20 mL with dichloromethane, and washed with three 10-mL portions of 5% hydrochloric acid. The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Preparative TLC of the residue on silica gel (three 1-mm plates) with 5% methanol in dichloromethane as eluent yielded 203 mg (82%) of **17**, an analytical sample of which was obtained by recrystallization from dichloromethane–hexane: mp >300 °C; $[\alpha]^{25}_D$ +254° (c 1.68, $CHCl_3$); IR ($CHCl_3$) 3560, 1745, 1715, 1685, 1635, 1590 cm^{-1}. Anal. ($C_{27}H_{32}O_{10}$) C, H.

16-Oxoverrucarin A (18). A procedure similar to that described for the preparation of **17** provided, from 120 mg of 16-hydroxyverrucarin A (**15**), 75 mg (63%) of **18**: mp >300 °C; $[\alpha]^{28}_D$ +170° (c 4.28, $CHCl_3$); IR ($CHCl_3$) 3560, 1715, 1635, 1590 cm^{-1}. Anal. ($C_{27}H_{32}O_{10}$) C, H.

(18) Yagi, H.; Takker, D. R.; Hermandez, O.; Koreeda, M.; Jerina, D. M. *J. Am. Chem. Soc.* **1977**, *99*, 1604.
(19) Kindly provided by Dr. James Schmidt, University of Virginia.

Reduction of 8-Oxoverrucarin A (17). A solution of 50 mg (0.10 mmol) of **17** in 0.65 mL of THF and 0.35 mL of absolute ethanol was cooled to −5 °C in an ice–water–sodium chloride bath. Sodium borohydride (11 mg, 0.29 mmol) was added in one portion and the mixture was stirred for 90 min with the temperature maintained between −5 and 0 °C. The mixture was then poured into 25 mL of water and extracted with three 25-mL portions of ethyl acetate. The combined organic layers were dried (magnesium sulfate) and concentrated in vacuo. Preparative TLC of the residue on silica gel (one 1-mm plate) with ethyl acetate as eluent afforded 28 mg (56%) of 8α-hydroxy-9,10-dihydroverrucarin A (**19**) and 18 mg (36%) of 8α-hydroxyverrucarin A (**20**).

Recrystallization from dichloromethane–hexane provided an analytical sample of **19**: mp >300 °C; $[\alpha]^{26}_D$ +141° (c 2.76, $CHCl_3$); IR ($CHCl_3$) 3520, 1715, 1635, 1590 cm^{-1}. Anal. ($C_{27}H_{36}O_{10}\cdot 0.5H_2O$) C, H.

Recrystallization from ethyl acetate–hexane provided an analytical sample of **20**: mp >300 °C; $[\alpha]^{28}_D$ +139° (c 2.94, Me_2SO); IR (KBr) 3460, 1710, 1635, 1590 cm^{-1}. Anal. ($C_{27}H_{34}O_{10}$) C, H.

When this reduction was carried out at −35 °C, **20** was isolated in 62% yield, **19** was isolated in 5% yield, and 7% of **17** was recovered unchanged.

Hydrolysis of 8α-Hydroxyverrucarin A (20). A procedure similar to that described for the hydrolysis of **14** gave, from 198 mg of **20**, 76 mg (70%) of 4β,8α,15-trihydroxy-12,13-epoxytrichothecene (**21**): mp 177–179 °C; $[\alpha]^{26}_D$ −71.3 °C (c 3.49, CH_3OH); IR (KBr) 3280. Anal. ($C_{15}H_{22}O_5$) C, H.

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Supplementary Material Available: Full NMR data (Table II) for compounds **7**, **9–12**, **14**, **15**, and **17–21** (6 pages). Ordering information is given on any current masthead page.

1-[(Ethoxyamino)methyl]-1,3,4,5-tetrahydro-7,8-dimethoxy-2-benzoxepins: A New Class of Antianaphylactic Agents

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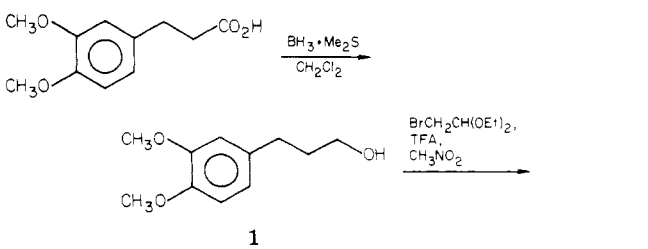
The synthesis and biological activity in the rat passive cutaneous anaphylaxis (PCA) test of a new class of compounds, 1-[(ethoxyamino)methyl]-1,3,4,5-tetrahydro-7,8-dimethoxy-2-benzoxepins, are reported. These compounds are synthesized from the adduct of 1-(bromomethyl)-1,3,4,5-tetrahydro-7,8-dimethoxy-2-benzoxepin and ethylene glycol or chloroethanol. The influence of the amine function on activity in the rat PCA is discussed. Aryl- or heteroarylpiperazines favor activity with this class of compounds.

In the past decade a number of compounds have been synthesized which inhibit the anaphylactic response in the rat passive cutaneous anaphylaxis (PCA) test. The prototype compound, disodium cromoglycate,[1] which is not absorbed orally, was followed by a number of orally active agents[2] which were identified by the rat PCA screen.[3] We now report a new class of compounds, the 1-ethoxy-1,3,4,5-tetrahydro-2-benzoxepins, which are active antiallergic agents.

Scheme I



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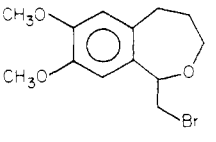

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