partitioned between Et_2O and H_2O, and acidified with aqueous HCl. The Et_2O extract was washed with dilute HCl and then H_2O and finally dried over $MgSO_4$. Evaporation of the Et_2O extract gave 8-4 enriched with **8-4B** (16.2 g, 0.040 mol), which was dissolved in boiling EtOH (110 mL) and treated with cinchonidine (11.9 g, 0.04 mol) dissolved in boiling EtOH (110 mL). The solid that separated was recrystallized 10 times from DMF, then partitioned between H_2O and Et_2O, and acidified with dilute HCl. The Et_2O extract was washed with dilute HCl and then with H_2O and finally dried over $MgSO_4$. Evaporation of the Et_2O extract gave **8-4B**: mp 172–173.5 °C; $[\alpha]^{24}_D$ −18.6° (*c* 5, EtOH). Anal. ($C_{20}H_{24}Cl_2O_4$) C, H.

(+)-2-Butyl-6,7-dichloro-2-cyclopentyl-2,3-dihydro-5-hydroxy-1*H*-inden-1-one (6-4A). Using the procedure for the preparation of **6-2A** but substituting an equimolar amount of **8-4A** for the **8-2A**, we obtained **6-4A**, which was used in the next step without purification.

(−)-2-Butyl-6,7-dichloro-2-cyclopentyl-2,3-dihydro-5-hydroxy-1*H*-inden-1-one (6-4B). Employing the procedure described for the preparation of **6-2A** but substituting an equimolar amount of **8-4B** for **8-2A**, we obtained **6-4B**, which was used in the next step without purification.

(+)-4-[(2-Butyl-6,7-dichloro-2-cyclopentyl-2,3-dihydro-1-oxo-1*H*-inden-5-yl)oxy]butanoic Acid (8-13A). By following the procedure described for the preparation of **8-10A** but substituting an equimolar amount of **6-4A** for the **6-2A**, we obtained **8-13A**: mp 139–139.5 °C; $[\alpha]^{25}_D$ +18.4° (*c* 5, EtOH). Anal. ($C_{22}H_{28}Cl_2O_4$) C, H.

(−)-4-[(2-Butyl-6,7-dichloro-2-cyclopentyl-2,3-dihydro-1-oxo-1*H*-inden-5-yl)oxy]butanoic Acid (8-13B). By following the procedure described for the preparation of **8-10A** but substituting an equimolar amount of **6-4B** for **6-2A**, we obtained **8-13B**: mp 139–139.5 °C; $[\alpha]^{25}_D$ −17.7° (*c* 5, EtOH). Anal. ($C_{22}H_{28}Cl_2O_4$) C, H.

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Structural Modifications of Anguidin and Antitumor Activities of Its Analogues

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Approximately 60 derivatives of anguidin were prepared for evaluation of antitumor activities. Positions 3, 4, 8–10, and 15 were modified, and the resultant derivatives were screened against P-388 leukemia. It was found that introduction of the C3-keto and C3,C8-diketo groups markedly improved the antileukemic activity, whereas epoxidation of the C9–C10 double bond or oxidation of the C15 position diminished its activity. Selected derivatives were further tested in the L1210, B16, Lewis lung, Colon 36, and Colon 38 tumor lines. Among these compounds, 4β,15-diacetoxyscirpene-3,8-dione (**54**) and 4β-(chloroacetoxy)-15-acetoxyscirpene-3,8-dione (**55**) were found to be most active in various tumors. Inhibitory action of several analogues on protein synthesis was also examined using H-HeLa cells.

Anguidin (4β,15-diacetoxyscirpen-3α-ol, **1**) is a fungal metabolite produced by *Fusarium equiseti*.[1] It belongs to the family of trichothecenes, many of which have been shown to have cytotoxic and antitumor activities.[2] Anguidin shows marked activities against P-388 and L1210 leukemias;[3] however, it is only marginally active against B16 melanoma and Lewis lung tumor. Phase I and Phase II clinical studies have been carried out with limited success.[4] Trichothecenes are inhibitors of eukaryotic protein synthesis;[5] more specifically, anguidin has been reported to inhibit the initiation of protein synthesis at low concentrations (e.g., 5 μg/mL) in HeLa cells, and at high concentrations (100 μg/mL), it behaves as an inhibitor of polypeptide chain elongation.[6]

Although extensive work on the modification of anguidin was carried out at the time of its discovery,[7,8] little has been

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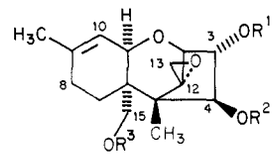
0022-2623/82/1825-0579$01.25/0

Chart I. Structures of Scirpene-3α,4β,15-triol (2) and Esters[a]



	R¹	R²	R³		R¹	R²	R³
1	H	Ac	Ac	12	H	BrAc	BrAc
2	H	H	H	13	H	Cr	Cr
3	ClAc	H	H	14	H	Ma	Ma
4	H	ClAc	H	15	THP	Ac	Ac
5	H	H	ClAc	16	THP	ClAc	ClAc
6	ClAc	H	ClAc	17	H	H	Cr
7	ClAc	ClAc	ClAc	18	H	Cr	H
8	THP	H	Ac	19	H	H	Ma
9	THP	H	H	20	H	Ma	H
10	H	ClAc	Ac	21	H	ClAc	Ma
11	H	ClAc	ClAc				

[a] Ac = acetyl; ClAc = chloroacetyl; THP = 2-tetrahydropyranyl; BrAc = bromoacetyl; Cr = crotonyl; Ma = methacryloyl.

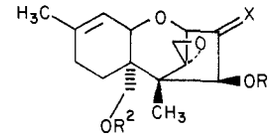
reported on the antitumor activities of the resultant derivatives.[9] We became interested in a systematic modification of anguidin in search of analogues having higher antitumor activities. Here we present some of our synthetic work and a summary of the observed structure–activity relationships.

Chemistry. The analogues prepared include compounds resulting from modifications at C3, C4, C8, C9–C10, or C15, as well as various esters of scirpentriol (**2**). They are grouped accordingly.

For characterization of new derivatives, NMR was used most extensively since this class of compounds exhibits well-defined NMR patterns.[2] The most obvious signals are the AB quartet around δ 2.9 (J = 4 Hz) due to the epoxide methylene protons. The C14 and C16 methyl groups appear as singlets approximately at δ 0.8 and 1.7, respectively. The latter peak is broadened due to allylic coupling to the C10 proton and occasionally a small coupling constant (J = 1 Hz) is observable. The chemical shifts of the C3 and C4 protons vary considerably, depending on whether these centers are acylated (approximately δ 4.1–5.2 for C3 H and δ 4.5–5.8 for C4 H). The C15 methylene group usually appears as an AB quartet (J = 12–13 Hz) around δ 4.0 or less frequently as a singlet. The vinylic proton appears consistently around δ 5.5 as a broad doublet or a quartet of doublets (J = 1 and 6 Hz). When it is a part of an enone system, it is shifted downfield approximately by 1 ppm. The C11 proton gives rise to a broad doublet around δ 4.0–4.5, but it is often buried under other peaks. The C7,C8 methylene protons appear as an envelope around δ 2.0. In Table I are listed the characteristic NMR signals of the new derivatives.

For some compounds, a poor elemental analysis was obtained, mainly because of their instability. These compounds are indicated with an asterisk. The spectroscopic data, however, indicated that they are more than 95% pure, and we believe that the biological data obtained on these compounds are valid for the purpose of this study.

Chart II. C3-Modified Anguidin Derivatives[a]



	R¹	R²	X
22	Ac	Ac	O
23	Ac	Ac	NOH
24	H	Ac	NOH
25	Ac	Ac	NNHTs[a]
26	ClAc	Ac	O
27	ClAc	ClAc	O
28	Ma	Ma	O
29	ClAc	Ma	O

[a] For abbreviations, see Chart I; Ts = tosyl.

Esters of Scirpenetriol. In our biotransformation program, it was found that 15-acetoxyscirpene-3α,4β-diol was more active against P-388 leukemia than anguidin.[10] This finding prompted us to investigate other esters of scirpenetriol. Initially, the acetyl, propionyl, and butyryl esters were prepared by acylation of scirpenetriol (**2**), followed by chromatographic separation or selective hydrolysis.[11] Chloroacetyl esters **3**–**7** (Chart I) were also prepared by this method.

It became evident, however, from the in vivo antitumor data of these derivatives that 15-acyl and 4β,15-diacyl derivatives were more active than other positional isomers and homologues. In order to prepare these compounds selectively, a scheme involving the initial protection of the 3α-hydroxy group of anguidin as a THP ether was developed. Thus, the acetyl groups of the THP ether of anguidin (**15**) were hydrolyzed either to diol **9** with NaOH or to mono-ol **8** with NH_4OH. From diol **9** were prepared compounds **11**–**14** by acylation, followed by cleavage of the THP group. Compound **10** was obtained from **8** by treatment with chloroacetic anhydride and deprotection.

Since many of the biologically active trichothecenes, such as baccharin[12] and rorridin E,[13] contain unsaturated esters at the C4 and/or C15 positions, α,β-unsaturated esters of scirpenetriol were prepared by acylating **9** with methacryloyl chloride or crotonyl chloride. Even with a large excess of an acylating agent, the acylation was sluggish, and monoesters **17**–**20** were obtained, together with diesters **13** and **14**.

Modifications at C3. It was found that the C3 hydroxy group in anguidin could be easily oxidized by the method of Swern[14] to give ketone **22**, which was first reported as a minor product in the CrO_3 oxidation of anguidin.[7] Since it exhibited markedly improved activities (Tables II and IV), several derivatives of this ketone were prepared by standard methods. Thus, treatment with hydroxylamine gave oxime **23** as a 2:1 mixture of syn and anti isomers in 49% yield.[7] Apparently, a partial hydrolysis took place in this reaction, and a 3:1 mixture of *syn*- and *anti*-oximes of 15-acetoxy-4β-hydroxyscirpen-3-one (**24**) was also isolated in 15% yield. Reaction of **22** with *p*-toluenesulfonylhydrazide gave hydrazone **25** in 76% yield.

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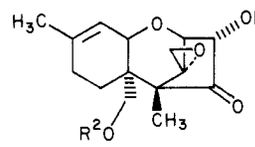
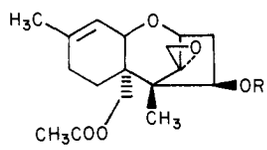
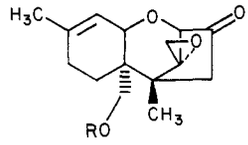
Chart III. C3-C4 Modified Anguidin Derivatives[a]



30, $R^1 = R^2$ = ClAc
35, $R^1 = CH_3SO_2$; R^2 = Ac



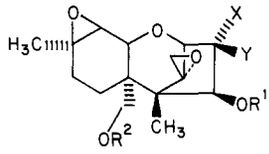
31, R = Ac
32, R = H



33, R = H
34, R = Ac

[a] For abbreviations, see Chart I.

Chart IV. C9-C10 β-Epoxides



	R^1	R^2	X	Y
36	Ac	Ac	OH	H
37	Cr	Cr	OH	H
38	H	Cr	OH	H
39	Ac	Ac	-O-	

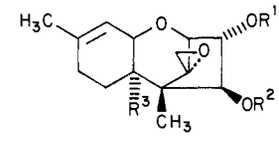
Oxidation of alcohols **10**, **11**, **14**, and **21** by the same method provided the corresponding ketones **26–29**. These C4-acyloxy C3-ketones were unstable to silica gel, possibly due to the epimerization at C4 or the keto–acyloxy exchange.[15] Thus, their isolation was effected by crystallization from the product mixture. The actual yields are presumed to be higher than the isolated yields. The C4 ketone (**30**) was prepared from **6**. In order to examine the significance of the C3 hydroxy group of anguidin, diacetoxyverrucarol, **31**, was prepared by acetylation of verrucarol, which in turn was obtained by hydrolysis of verrucarin A.[16] Monoacetate **32** was also isolated from the acetylation experiment. Ketones **33–35** were prepared according to the methods described in the original paper.[7]

C9–C10 Modifications. Baccharin, which is a potent antitumor trichothecene, contains a C9–C10 β-epoxide.[12] To test the effect of epoxidation in nonmacrocyclic trichothecenes, we prepared compounds **36–38** from the corresponding alkene by a treatment with *m*-chloroperoxybenzoic acid. Epoxide **36** was further oxidized by the previously mentioned method to give ketone **39**.

The β orientation of the newly formed epoxides was assigned on the basis of their NMR spectra. For example, in compound **39** the C10 proton signal appeared at δ 3.12 with a coupling constant of 6 Hz, indicating that the C10 and C11 protons are cis to each other.[17,18]

C15 Modifications. For the modification of the C15 position, diol **9** appeared to be an appropriate starting material, and pyridinium chlorochromate (PCC)[19] was found to selectively oxidize the C15 alcohol in **9** to give aldehyde **40**. A subsequent cleavage of the THP group provided aldehyde **41** whereas acetylation of **40**, followed by deprotection, gave **43**. Treatment of **42** with semicarbazide afforded **44** and, after deprotection, **45**. Neutral $KMnO_4$ in aqueous acetone oxidized **40** to acid **46**.

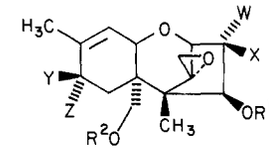
Chart V. C15-Modified Anguidin Derivatives[a]



	R^1	R^2	R^3
40	THP	H	CHO
41	H	H	CHO
42	THP	Ac	CHO
43	H	Ac	CHO
44	THP	Ac	$CH=NNHCONH_2$
45	H	Ac	$CH=NNHCONH_2$
46	H	H	CO_2H

[a] For abbreviations, see Chart I.

Chart VI. C8-Modified Anguidin Derivatives[a]



	R^1	R^2	W	X	Y	Z
47	H	Ac	OH	H	H	O-iVal
48	Ac	Ac	OH	H	OH	H
49	ClAc	Ac	OH	H	OH	H
50	Ac	Ac	OH	H	-O-	
51	Ac	Ac	OH	H	H	OH
52	Ac	Ac	OH	H	H	Br
53	Ac	Ac	OH	H	Br	H
54	Ac	Ac	-O-		-O-	
55	ClAc	Ac	-O-		-O-	
56	H	H	O-THP	H	O-THP	H
57	Ma	Ac	O-THP	H	O-THP	H
58	Ma	Ac	OH	H	OH	H
59	Ma	Ac	-O-		-O-	
60	Ma	Ma	OH	H	OH	H
61	Ma	Ma	-O-		-O-	

[a] For abbreviations, see Chart I; iVal = isovaleryl.

C8 Modifications. T2 toxin (**47**) as well as several other trichothecenes, contains an oxygen functionality at C8, and it exhibits significant antitumor activities.[2] Thus, the functionalization of this position in **1** was attempted. For example, the SeO_2 oxidation of anguidin (**1**) afforded 4β,15-diacetoxyscirpene-3α,8β-diol (**48**), together with minor isomers. The coupling constant between the C8 proton and one of the C7 protons of **48** was determined to be 8 Hz. This indicated that these protons were in the axial–axial relationship. Thus, the newly introduced hydroxy group was in the β configuration. Our result is consistent with the observation made by Jarvis and coworkers in the SeO_2 oxidation of verrucarin A.[20] It is also consistent with a molecular model which indicates that the β face of the molecule is sterically less congested than the α face. Compound **49** was similarly prepared from diester **10** in 42% yield.

Treatment of allylic alcohol **48** with PCC gave enone **50**. The same transformation could be effected by MnO_2 in

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Table I. ^{1}H NMR Spectra of Anguidin Derivatives[a]

no.	C_2	C_3	C_4	C_{10}	C_{13}	C_{15}
1	3.67 (d; 5)	4.19 (2 d; 3, 5)	5.20 (d; 3)	5.54 (d; 5)	2.77 + 3.06 (AB; 4)	3.96 + 4.18 (AB; 12)
2	3.53 (d; 5)	4.12 (2 d; 3, 5)	4.49 (d; 3)	5.50 (d; 5)	2.72 + 2.98 (AB; 4)	3.47 + 3.73 (AB; 12)
3	3.84 (d; 5)	4.98 (2 d; 3, 5)	4.70 (m)	5.47 (d; 5)	2.80 + 3.07 (AB; 4)	3.55 + 3.78 (AB; 12)
4	3.63 (d; 5)	4.28 (2 d; 3, 5)	5.70 (d; 3)	5.58 (d; 5)	2.75 + 3.02 (AB; 4)	3.58 + 3.86 (AB; 12)
5	3.62 (d; 5)	4.2-4.4 (m)	4.2-4.4 (m)	5.50 (d; 5)	2.74 + 3.02 (AB; 4)	4.02 + 4.30 (AB; 12)
6	3.88 (d; 5)	5.02 (2 d; 3, 5)	4.47 (2 d; 3, 5)	5.47 (d; 5)	2.80 + 3.07 (AB; 4)	4.06 + 4.26 (AB; 12)
7	3.92 (d; 5)	5.24 (2 d; 3, 5)	5.92 (d; 3)	5.50 (d; 5)	2.81 + 3.09 (AB; 4)	4.0-4.4 (m)
8	3.70 (d; 5)	3.8-4.4 (m)	3.8-4.4 (m)	5.48 (d; 6)	2.77 + 3.02 (AB; 4)	3.96 + 4.20 (AB; 13)
9	3.4-4.2 (m)	3.4-4.2 (m)	4.39 (d; 3)	5.50 (m)	2.75 + 3.00 (AB; 4)	3.42-4.16 (m)
10	3.73 (d; 5)	3.9-4.3 (m)	5.36 (d; 3)	5.56 (d; 5)	2.81 + 3.60 (AB; 4)	4.00-4.19 (AB; 13)
11	3.81 (d; 5)	4.0-4.4 (m)	5.34 (d; 4)	5.53 (d; 5)	2.79 + 3.07 (AB; 4)	4.12 + 4.30 (AB; 12)
12	3.71 (d; 5)	4.0-4.3 (m)	5.33 (m)	5.53 (d; 5)	2.78 + 3.06 (AB; 4)	4.0-4.3 (m)
13	3.67 (d; 5)	4.05-4.2 (m)	5.16 (d; 3)	5.52 (d; 5)	2.75 + 3.02 (AB; 4)	4.00 + 4.20 (AB; 12)
14	3.71 (d; 5)	4.1-4.3 (m)	5.14 (d; 3)	5.5-5.7 (m)	2.79 + 3.06 (AB; 4)	4.07 + 4.27 (AB; 12)
15	3.72 (d; 5)	3.9-4.4 (m)	5.64 (d; 3)[b] 5.70 (d; 3)	5.5 (m)	2.75 + 3.03 (AB; 4)	4.06 + 4.30 (AB; 13)
16	3.81 (d; 4)	4.1-4.6 (m)	5.77 (d; 3) 5.82 (d; 3)	5.5 (m)	2.81 + 3.09 (AB; 4)	4.1-4.6 (m)
17	3.68 (d; 6)	4.2-4.4 (m)	4.2-4.4 (m)	5.52 (d; 6)	2.78 + 3.06 (AB; 4)	3.97 + 4.28 (AB; 12)
18	3.66 (d; 4)	4.2 (m)	5.56 (m)	5.56 (m)	2.75 + 3.03 (AB; 4)	3.60 + 3.82 (AB; 12)
19	3.61 (d; 4)	4.2-4.4 (m)	4.28 (m)	5.50 (d; 5)	2.73 + 3.01 (AB; 4)	3.92 + 4.28 (AB; 13)
20	3.6-3.9 (m)	4.1-4.4 (m)	5.5-5.8 (m)	5.5-5.8 (m)	2.82 + 3.10 (AB; 4)	3.6-3.9 (m)
21	3.63 (m)	4.0-4.5 (m)	5.37 (d; 3)	5.5-5.7 (m)	2.84 + 3.11 (AB; 4)	4.0-4.5 (m)
22	3.56 (s)		5.96 (s)	5.49 (d; 5)	2.96 + 3.18 (AB; 4)	4.09 + 4.25 (AB; 12)
23	4.60 (s)[c] 4.10 (s)		6.40 (s) 6.74 (s)	5.48 (m)	2.88 + 3.16 (AB; 4) 2.88 + 3.19 (AB; 4)	4.16 (s)
24	4.59 (s)[c] 4.07 (s)		4.88 (s) 5.16 (s)	5.46 (d; 5)	2.91 + 3.17 (AB; 4)	3.94 + 4.24 (AB; 12)
25	3.96 (s)[c] 4.12 (s)		6.16 (s)	5.30 (d; 5) 5.16 (d; 5)	2.81 + 3.09 (AB; 4) 2.80 + 3.05 (AB; 4)	4.00 (s)
26	3.56 (s)		6.10 (s)	5.50 (d; 5)	2.98 + 3.19 (AB; 4)	4.10 + 4.25 (AB; 12)
27	3.57 (s)		5.96 (s)	5.55 (d; 5)	2.99 + 3.28 (AB; 4)	4.32 (s)
28	3.47 (s)		5.72 (s)	5.44 (d; 5)	2.98 + 3.16 (AB; 4)	4.15 + 4.30 (AB; 12)
29	3.57 (s)		5.79 (s)	5.48 (d; 5)	3.00 + 3.20 (AB; 4)	4.27 (s)
36	3.71 (d; 5)	3.20 (2 d; 3, 5)	5.09 (d; 3)	3.18 (d; 5)	2.75 + 3.15 (AB; 4)	3.97 + 4.19 (AB; 12)
37	3.80 (d; 5)	4.05-4.2 (m)	5.11 (d; 3)	4.05-4.2 (m)	2.73 + 3.02 (AB; 4)	4.02 + 4.24 (AB; 13)
38	3.76 (d; 4)	4.15-4.4 (m)	4.15-4.4 (m)	3.21 (d; 5)	2.73 + 3.11 (AB; 4)	3.99 + 4.16 (AB; 13)
39	3.72 (s)		5.77 (s)	3.12 (d; 5)	2.92 + 3.22 (AB; 4)	4.20 (s)
40	3.72 (d; 5)	4.12 (2 d; 3, 5)	4.33 (m)	5.62 (d; 5)	2.81 + 3.09 (AB; 4)	9.70 (s)
41	3.68 (d; 5)	4.29 (m)	4.29 (m)	5.63 (d; 5)	2.81 + 3.08 (AB; 4)	9.68 (s)
42	3.81 (d; 5)	4.12 (2 d; 3, 5)	5.59 (d; 3)	5.65 (m)	2.83 + 3.07 (AB; 4)	9.75 (s)
43	3.85 (d; 5)	4.20 (3 d; 2, 3, 5)	5.01 (d; 3)	5.68 (d; 5)	2.85 + 3.12 (AB; 4)	9.70 (s)
44	3.80 (d; 5)	4.29 (2 d; 3, 5)	5.62 (d; 3)	5.58 (m)	2.82 + 3.08 (AB; 4)	7.00 (s)
45	3.69 (d; 5)	4.25 (2 d; 3, 5)	5.26 (d; 3)	5.58 (m)	2.82 + 3.09 (AB; 4)	7.36 (s)
46	3.56	4.15 (m)	4.59 (d; 3)	5.22 (d)	2.73 + 2.96 (AB; 4)	
48	3.64 (d; 4)	3.8-4.2 (m)	4.95 (d; 3)	5.47 (d; 5)	2.76 + 3.01 (AB; 4)	3.82 + 4.12 (AB; 12)
49	3.75 (d; 5)	4.23 (2 d; 3, 5)	5.28 (d; 3)	5.62 (d; 5)	2.85 + 3.13 (AB; 4)	3.96 + 4.14 (AB; 13)
50	3.81 (d; 5)	4.25 (2 d; 3, 5)	5.11 (d; 3)	6.64 (d, q; 1, 6)	2.84 + 3.12 (AB; 4)	4.18 (s)
51	3.71 (d; 5)	4.1-4.3 (m)	5.36 (d; 3)	5.70 (d; 5)	2.82 + 3.08 (AB; 4)	4.25 + 4.40 (AB; 12)
52	3.75 (d; 5)	4.20 (2 d; 3, 5)	5.25 (d; 3)	5.89 (d; 5)	2.82 + 3.12 (AB; 4)	4.24 + 4.40 (AB; 12)
54	3.56 (s)		5.85 (s)	6.48 (d, q; 1, 6)	2.84 + 3.12 (AB; 4)	3.97 + 4.35 (AB; 12)
55	3.66 (s)		6.03 (s)	6.55 (d, q; 1, 6)	3.02 + 3.23 (AB; 4)	4.05 + 4.39 (AB; 13)
58	3.74 (d; 5)	3.8-4.2 (m)	5.12 (d; 3)	5.60 (d; 5)	2.82 + 3.09 (AB; 4)	3.92 + 4.19 (AB; 13)
59	3.64 (s)		5.96 (s)	6.52 (d, q; 1, 5)	3.01 + 3.20 (AB; 4)	4.12 + 4.42 (AB; 13)
60	3.76 (d; 5)	4.05-4.25 (m)	5.02 (d 3)	5.55-5.7 (m)	2.85 + 3.01 (AB; 4)	4.00 + 4.25 (AB; 13)
61	3.66 (s)		5.81 (s)	6.53 (d; 5)	3.02 + 3.20 (AB; 4)	4.30 + 4.50 (AB; 12)

[a] Recorded at 100 MHz in $CDCl_3$ with Me_4Si as internal standards. Chemical shifts (in parts per million), followed by multiplicity and coupling constants (J, in hertz) in parentheses. [b] A diastereoisomeric mixture due to the THP group. [c] Two sets of resonances were observed due to syn-anti isomerism.

acetone; however, PCC was more efficient. The epimer of **48**, 8α-hydroxy compound **51**, is a naturally occurring trichothecene called neosolaniol.[21] Interconversion of anguidin to neosolaniol was accomplished by the diisobutylaluminum hydride (DIBAH) reduction of enone **50**. The coupling constant for the C7 and C8 protons in **51**, which was produced in 38% yield, was observed to be 5 Hz. This reduction also gave **48** in 18% yield.

Allylic bromination of anguidin with *N*-bromosuccinimide (NBS) gave a mixture of C8 α- and β-bromo derivatives (**52** and **53**), the latter of which predominated. The stereochemistry was again determined by the coupling constant of the C7 and C8 protons. Thus, the C8 proton of **52** gave rise to a doublet at δ 4.82 with a coupling constant of 6.2 Hz, whereas the same proton in **53** exhibited a doublet of doublets at δ 4.53 with coupling constants of 5.8 and 11 Hz. Testing was carried out only on the minor isomer (**52**), since the major isomer could not be isolated pure even after extensive chromatography. Hydrolysis of the crude NBS bromination mixture, catalyzed by a silver salt, provided alcohols **48** and **51** in approximately a 2:1 ratio.

In view of the improved activities of C3-keto derivatives as mentioned earlier, oxidation of 3α,8β-diols was carried out using the Me_2SO–TFAA method. Compounds **54** and

(21) Ishii, K.; Sakai, K.; Ueno, Y.; Tsunoda, H.; Enomoto, M. *Appl. Microbiol.* 1971, *22*, 718.

Table II. P-388 Leukemia Activities

no.	P-388 antileukemic act.[a] opt dose, (mg/kg)/ injectn	max T/C, % (survivors/total)[e]	act. index[b]
	Ester Series		
1	~1.6	150-219	1.00
2	6.4	183	0.76
3	6.4	194	1.03
4	6.4	156	0.83
5	8.0	198	1.00
6	6.4	161	*c*
7	6.4	206	*c*
8		(-)[d]	
9		(-)	
10	1.6	222	1.17
11	6.4	212	1.13
12*	1.6	144	0.81
13	6.4	167	1.00
14	1.6	133	0.73
15		(-)	
16	1.6	139	0.74
17	3.2	222	1.17
18	12.8	156	0.80
19	0.4	225	1.20
20*	12.8	139	0.86
21*	0.8	194	1.20
	C3 Modifications		
22	0.8	222	1.42
23	25.6	181	0.96
24	25.6	163	0.87
25	25.6	125	0.66
26	0.8	233	1.23
27	6.4	188	1.00
28	1.6	233	1.31
29	0.8	211	1.23
	C9,C10 β-Epoxides		
36		(-)	
37*	25.6	138	0.68
38	12.8	183	0.94
39*	12.8	125	0.63
	C15 Modifications		
40		(-)	
41		(-)	
42		(-)	
43		(-)	
44		(-)	
45*	6.4	156	0.88
46		(-)	
	C8 Modifications		
48	0.8	167	0.97
49	0.8	167	0.94
50*	1.6	165	1.10
51*	1.6	156	0.85
52	1.6	188	0.94
54	1.6	306 (2/6)	1.67
55*	1.6	270 (1/6)	1.35
58	6.4	138	0.69
59	1.6	247	1.31
60		(-)	
61*	3.2	222	1.21

[a] For the experimental parameters, see Table III. [b] Maximum T/C of analogue/maximum T/C of anguidin in the same experiment. [c] Anguidin was not adequately evaluated in this experiment, so a fair comparison of the relative drug effect cannot be made. [d] Inactive (i.e., T/C < 125). [e] "Cures" are mice surviving to day 30 post-implant.

55 were thus prepared from **48** and **49**, respectively. Like C3-keto derivatives, these diketones were unstable to silica gel, and their isolation was best carried out by crystallization from the product mixture.

For the preparation of C3,C8-diketo derivatives containing α,β-unsaturated esters, a scheme involving the exchange of the ester groups in **48** was followed. Thus, the C8-hydroxy group in **48** was protected as a THP ether, and the resulting diacetate was hydrolyzed to diol **56**. A selective acylation of the C15 hydroxy group was effected by treating **56** with 1 equiv of acetyl chloride at 0 °C. The resulting compound was subsequently acylated with methacryloyl chloride to give **57**, which, after cleavage of the THP groups, provided diol **58**. Treatment of **56** with excess methacryloyl chloride, followed by cleavage of the THP groups, gave **60** in 76% yield. Oxidation of **58** and **60** by the Me_2SO–TFAA method gave diketones **59** and **61** in high yields.

Biological Results and Discussion

Antitumor Effects. The antitumor activities of the analogues against P-388 lymphocytic leukemia cell growth are listed in Table II. The analogues were tested in a standard manner.[22] The methods of assay are detailed in Table III and in the Experimental Section. The data are a compilation of the results from a series of separate experiments where anguidin was always included as a standard. To facilitate a direct comparison of the data from different experiments, "an activity index", the ratio of the maximum T/C of analogue and the maximum T/C of anguidin in the same test, is also listed in the tables. Compounds **30–35**, which do not appear in the tables, were inactive (T/C < 125) up to doses of 0.8 mg/kg, although several of these compounds were tested at much higher doses as well. Some of the selected analogues were further tested against L1210 lymphoid leukemia and B16 melanoma, and the results are shown in Table IV.

It can be seen from these tables that several analogues of markedly enhanced activity were obtained by subtle structural modifications. Compounds **10**, **17**, **19**, **21***, **22**, **26**, **28**, **29**, **54**, **55***, **59**, and **61*** fall into this category as far as their activities in the P-388 screen are concerned. Their potencies are, in general, comparable to that of anguidin. Among these, compounds **22**, **54**, and **55** were the most active. Derivatives **10**, **22**, **54**, and **55** were also more active than the parent compound against L1210 leukemia growth (Table IV). Diketo derivative **54** again exhibited the highest activity index. Against B16 melanoma, most analogues tested behaved similarly to anguidin. Although one of the ester derivatives, **17**, originally exhibited a high activity against this tumor growth, repeated trials failed to reproduce this activity. Compound **54** reproducibly showed a higher activity than anguidin, but the advantage was slight in this solid tumor test.

Analysis of the P-388 data suggests several trends in structure–activity relationships. They are discussed under each class of derivatives.

Esters of Scirpenetriol. The C15-monoesters and C4,C15-diesters, such as **5**, **10**, **11**, **17**, **19**, and **21**, are quite active. This observation parallels the previous results obtained with various acetates of scirpentriol.[23] Among the C4,C15-diesters, chloroacetates **10**, **11**, and **21** show the highest activities.[24] For incorporation of α,β-unsaturated

(22) Geran, R. I.; Greenberg, N. H.; MacDonald, M. D.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*, 8.
(23) Claridge, C. A.; Schmitz, H.; Bradner, W. T. *Cancer Chemother. Rep.* **1979**, *2*, 181.
(24) For example, 4β,15-bis(propionyloxy)scirpen-3α-ol, 4β,15-bis(butyroyloxy)scirpen-3α-ol, and 4β,15-bis(valeroyloxy)scirpen-3α-ol gave an activity index of 0.77, 0.81, and 0.77, respectively, in the P-388 assay.[11] This series also indicates that lengthening the ester chains does not improve the P-388 activity.

Table III

tumor and site of implant	host mouse strain[a]	group size	level of inoculum	drug treat. schedule	criteria for act.
P-388, ip	BDF_1 or CDF_1	6	10^6 cells	qd 1-9	T/C ≥ 125%
L1210, ip	BDF_1 or CDF_1	6	10^5 or 10^6 cells[b]	qd 1-9	T/C ≥ 125%
B16, ip	BDF_1	10	0.5 mL of a 10% tumor brei	qd 1-9	T/C ≥ 125%
Lewis lung, ip	BDF_1	10	10^6 cells	qd 1-9	T/C ≥ 125%
Colon 38, sc	BDF_1	10	tumor fragments, (~20 mg)	qdx5; d 15 and 26	T − C ≥ 8 days T/C ≥ 140%
Colon 36, sc	CDF_1	10	tumor fragments, (~20 mg)	qdx5; d 3 and 14	T − C ≥ 8 days T/C ≥ 140%

[a] Mice of both sexes were used. [b] 10^6 cells were used in the Bristol Laboratory experiments and 10^5 cells were used in the test conducted under the NCI auspices.

Table IV. L1210 and B16 Antitumor Activities of Anguidin Analogues[a]

	L1210 activity			B16 activity		
no.	OD, (mg/kg)/injecn	T/C, %	act. index[b]	OD, (mg/kg)/injecn	T/C, % (cures/total)[e]	act. index[b]
1	0.8-2.4	156-200	1.00	0.5-2.0	125-140	1.00
3					(-)[d]	
5				8.0	145	1.16
6					(-)	
10	0.8	171	1.09	0.8	131	c
11	6.4	175	0.96	8.0	146	1.12
17	0.8	143	0.91	3.0	252 (3/10)	1.85
				1.5	125	0.96
19	0.8	150	0.96	0.5	145	c
21*	1.6	167	0.84	2.0	152	1.09
22	0.8	200	1.09	2.0	133	1.02
26				0.8	147	c
29	2.4	183	0.92			
54	1.6	236	1.50	0.5	177 (1/10)	1.30
55*	2.0	250	1.25	1.5	145	1.02
59				1.5	145	1.02

[a-d] See corresponding footnotes in Table II. [e] "Cures" are mice surviving to day 60 post-implant.

esters, the C15 position again appears to be the most favored (e.g., **17**, **19**, and **21**).

C3 Modifications. Oxidation to the C3-keto compounds results in greatly enhanced activity with little or no loss in potency (**22**, **26**, **28**, and **29**). Oxidation of the C4 hydroxy group, on the other hand, did not provide active compounds (**30** and **35**). Derivatization of ketone **22** as an oxime or a *p*-toluenesulfonylhydrazone caused a loss in activity. Attachment of a THP group at the C3 hydroxy group inactivated the compond (**8**, **9**, **15**, and **16**). It appears that an oxygen functionality at C3 is important at least for potency, since diacetoxyverrucarol (**31**) and 15-acetoxyverrucarol (**32**) did not show antileukemic activity at some 40 times the minimum effective dose of anguidin. An oxygen functionality at C4 also appears to be important for activity, since compouds **33** and **34** are not active, whereas compound **22** is quite active.

C9–C10 Modifications. Epoxidation of the C9–C10 double bond gave analogues of diminished antileukemic activity, as exemplified by compounds **36**, **37***, and **38**. The diminished activity of these derivatives is in contrast to the observation made by Jarvis and co-workers, who reported improved activities for verrucarin A and B and roridin A expoxides.[20] Introduction of a ketone moiety in **36** results in a compound (**39***) that is barely active.

C15 Modifications. Table II indicates that C15-carboxaldehydes and C15-carboxylic acid are inactive at the doses tested (in each case the highest dose was 6.4 mg/kg). Only when the semicarbazone group is introduced does the compound exhibit modest activity. This might be a reflection of a rather stringent steric requirement at the C15 position.[25]

(25) Cundliffe, E.; Cannon, M.; Davies, J. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 30.

Table V. Madison 109 and Lewis Lung Antitumor Activities of Analogues **54** and **55**

	Madison 109[a]		Lewis lung[b]	
no.	OD, (mg/kg)/ injectn	T/C, %	OD, (mg/kg)/ injectn	T/C, %
1	0.8-1.6	95-122	0.8	203 (2/10)
54	0.8-1.6	124-144	1.2	203
1	0.8	112	0.4	135
55*	1.6	141	0.5	>353 (5/10)

[a] Qd 1-4 dosing following intraperitoneal implant of 0.5 mL of 2% (w/v) tumor brei. [b] Qd 1-9 dosing following intraperitoneal implant of 10^6 tumor cells. "Cures" are mice surviving to day 60 post-implant without evidence of tumor.

C8 Modifications. Analogues **51*** and **52** indcate that substitution at the C8α position with either a bromo or hydroxy group does not improve the antileukemic activity. Introduction of an enone system, as in **50***, improves the activity slightly compared to anguidin. Introduction of a C3,C8-diketo moiety, as in **54**, **55**, **59**, and **61**, on the other hand, resulted in greatly enhanced antileukemic activities. Although there are some variations in activity depending on the ester groups, these C3,C8-diketo analogues uniformly possess an activity index higher than 1.2.

Two analogues (**54** and **55**) from the last series were subjected to additional tumor testing (Table V). Unlike anguidin, both analogues were active against the intraperitoneally implanted M109 tumor, although the extent of activity was only modest. Neither analogue nor anguidin was active against subcutaneously implanted M109 (data not shown). Both analogues, however, were active against the Lewis lung tumor, especially **55** which displayed a dramatic effect (50% cures) in an experiment in which

Table VI. Colon 38 and 36 Antitumor Activities of Analogue 55

	Colon 38[a]			Colon 36[b]		
no.	OD, (mg/kg)/ injectn	T/C, % (cures/total)	T − C, days, 1.25 g	OD, (mg/kg)/ injectn	T/C, % (cures/total)	T − C, days, 0.75 g
1	4.4	159 (1/10)	29	2.8	141 (4/10)	11
55*	4.4	108	−3	4.4	178 (5/10)	22

[a] For the dosing schedule and other parameters, see Table III. "Cures" represent tumor-free mice as of day 106 post-implant. [b] For the dosing schedule and other parameters, see Table III. "Cures" represent tumor-free mice as of day 111 post-implant.

Table VII. Effects on Incorporation of L-[4,5-^{3}H]Leucine into Protein in Intact H-HeLa Cells

	% act. of control[a]					antileukemic effects[b]	
no.	0.1 concn: μg/mL	0.3 μg/mL	1.0 μg/mL	3.0 μg/mL	10 μg/mL	MED[c]	act. index
			Esters of Scirpenetriol				
10	55	35	31	ND[d]	ND	0.025	1.17
11	81	44	33	ND	ND	0.05	1.13
21*	80	53	36	ND	ND	0.1	1.20
1	80	55	26	ND	ND	0.1	1.00
19	91	44	33	ND	ND	0.1	1.20
			C3 Modification				
22	94	84	46	26	ND	0.0125	1.42
28	ND	96	ND	ND	49	0.1	1.31
39*	ND	ND	ND	ND	102	12.8	0.63
			C15 Modification				
41	72	ND	40	ND	13	6.4	*e*
43	44.5	ND	30	ND	16	6.4	*e*
			C8 Modification				
50*	ND	56	32	ND	15	0.4	1.10
48	ND	82	93	ND	30	0.1	0.97
51*	ND	89	84	32	29	0.8	0.85
58	ND	109	99	85	45	6.4	0.69
61*	ND	57	44	24	ND	0.1	1.21
54	ND	ND	89	64	26	0.5	1.67
59	ND	ND	89	68	36	0.4	1.31

[a] Percent activity relative to control at 8 min after introduction of drug. [b] P-388 activity; see Table II. [c] Minimum effective dose, (mg/kg)/injection, to produce T/C ⩾ 125. [d] ND = not determined. [e] Inactive.

anguidin was only just active. Two other analogues, **27** and **28**, were also evaluated in the Lewis lung tumor model (data not shown), and although active, they did not perform very differently from anguidin.

Based on its activity against Lewis lung tumor, compound **55** was evaluated against two colon tumor models, Colon 38 and 36 (Table VI). In terms of inhibition of tumor growth, the former is known to be quite sensitive to anguidin.[26] Mice bearing advanced stage Colon 38 (100–300 mg) implanted subcutaneously were treated with anguidin or **55**. The median time for anguidin-treated mice to have their tumors reach a size of 1.25 g, compared to untreated, tumor-bearing control mice (T − C, 1.25 g), was 29 days. Therapy with **55**, however, resulted in a T − C of −3 days, indicating that anguidin was active, whereas the analogue **55** was not. Against early stage (20–30 mg) subcutaneous Colon 36, both anguidin and **55** were active, with the latter having a slight therapeutic advantage, reflected in increased life span and tumor growth inhibition.

Effects on Protein Synthesis in Intact H-HeLa Cells. As previously described,[6] incorporation of L-[4,5-^{3}H]leucine into intact H-HeLa cells was examined at various concentrations of the trichothecene analogues and at various times after introduction of the drugs. Uptake of leucine by the H-HeLa cells at 8 min after introduction of the drug (the time-course plots indicate that there is an approximate 2–6 min lag phase), is indicated in Table VII as percent activity of control. The P-388 antiumor activities of these analogues are also listed in the same table.

Although all but one (**39**) of the analogues tested inhibited protein synthesis by more than 50% at a concentration of 10 μg/mL or lower, there seems to be no direct correlation between the P-388 activities and their protein-synthesis inhibitory effects. For example, 3,8-diketones **54** and **59** showed antileukemic activities superior to anguidin (**1**); however, they were at least an order of magnitude less potent than **1** as an inhibitor of protein synthesis in H-HeLa cells. Although they were inactive in the P-388 assay, C15 aldehydes **41** and **43**, on the other hand, were the most potent protein-synthesis inhibitors of the compounds tested.

If the comparison is limited, however, to the analogues more closely related in structure, some parallelism exists between the ID_{50}'s of protein synthesis inhibition, which can be estimated from the data given in Table VII, and the minimum effective doses against P-388 leukemia. This can be seen in a comparison of compounds **10** vs. **11** and **21**, **22** vs. **28**, **58** vs. **48**, or **61** vs. **54**.

Obviously, many factors are involved in the expression of antitumor activities of these drugs. The problem of transport into cells, for example, may be a significant factor. In addition, these analogues might have slightly different modes of action from each other.[6] Studies in cell-free protein-synthesizing systems and analysis of po-

(26) Corbett, T. H.; Griswold, Jr., D. P.; Roberts, B. J.; Peckham, J. C.; Schabel, Jr., F. M. *Cancer* **1977**, *40*, 2660.

lyribosome profiles may provide additional information concerning their mechanism of action.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. NMR spectra were obtained on a Varian HA-100 or XL-100 spectrometer using tetramethylsilane as internal standard. IR spectra were obtained on a Beckman 4240 spectrophotometer. Elemental analyses were performed by the Analytical Department of these laboratories. Column chromatography was run using either Mallinckrodt SilicAR CC-7 (100–200 mesh) or Merck silica gel 60 (230–400 mesh).

Chloroacetates of Scirpenetriol (3–7). Scirpenetriol (2) was prepared from anguidin as previously described.[7] Chloroacetic anhydride (10.75 g, 62.9 mmol) was added to a solution of 2 (7.0 g, 23.5 mmol) in 39 mL of 2,6-lutidine. After 18 h of stirring at room temperature, the solution was diluted with CH_2Cl_2 and washed with brine. The residue obtained after evaporation of the solvent was chromatographed on silica gel (gradient elution; initial eluent, CH_2Cl_2; final eluent, 5% MeOH–CH_2Cl_2). Fractions with R_f values of 0.35, 0.58, 0.68, 0.8, and 0.9 (Analtech silica gel GF plates developed with 4% MeOH–toluene) were collected. The fraction with the R_f value of 0.35 gave 640 mg (8%) of 15-(chloroacetoxy)scirpene-3α,4β-diol (5): mp 173–174 °C (EtOAc–Et_2O); IR (KBr) 3520, 3380, 1725, 1295, 1060 cm^{-1}. Anal. ($C_{17}H_{23}ClO_6$) C, H, Cl.

The fraction having an R_f value of 0.58 gave 480 mg (6%) of 3α-(chloroacetoxy)scirpene-4β,15-diol (3): mp 170–171 °C (EtOAc–Et_2O); IR (KBr) 3500, 1758, 1210, 1170, 1055 cm^{-1}. Anal. ($C_{17}H_{23}ClO_6$) C, H, Cl.

The fraction with the R_f value of 0.8 gave 1.50 g (15%) of 3α,15-bis(chloroacetoxy)scirpen-4β-ol (6): mp 161–162 °C (EtOAc–Et_2O); IR (KBr) 3480, 1765, 1735, 1295, 1200 cm^{-1}. Anal. ($C_{19}H_{24}Cl_2O_7$) C, H, Cl.

The fractions with the R_f values of 0.68 and 0.9 gave 205 mg (2%) of 4β-(chloroacetoxy)scirpene-3α,15-diol (4) and 95 mg (1%) of 3α,4β,15-tris(chloroacetoxy)scirpene (7), respectively which were characterized by NMR.

4β,15-Diacetoxy-3α-*O*-(2-tetrahydropyranyl)scirpene (15). A mixture of anguidin (12.81 g, 35 mmol), dihydropyran (17.5 mL, 189 mmol), and *p*-toluenesulfonic acid (70 mg, 0.35 mmol) in 150 mL of CH_2Cl_2 was stirred at room temperature for 2 h. The resulting solution was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ solution and brine. Drying over K_2CO_3 and removal of the solvent gave a colorless oil, which crystallized slowly from petroleum ether to give 11.30 g (72%) of solid: mp 93–94 °C; IR (KBr) 1746, 1249, 1080, 1040, 988 cm^{-1}. Anal. ($C_{24}H_{34}O_8$) C, H.

15-Acetoxy-3α-*O*-(2-tetrahydropyranyl)scirpen-4β-ol (8). To a solution of 4β,15-diacetoxy-3α-*O*-(2-tetrahydropyranyl)-scirpene (15; 31.4 g, 69.2 mmol) in 800 mL of MeOH and THF (1:1) was added 400 mL of 1.3 N NH_4OH solution. After the reaction was stirred for 3 days at room temperature, 10 mL of concentrated NH_4OH solution was added. Stirring was continued for an additional 4 days. The volume of the resulting solution was reduced to 500 mL. Extraction with CH_2Cl_2, washing with brine, and removal of the solvent gave 37 g of oil. Chromatography on silica gel (elution with 1% MeOH–CH_2Cl_2) gave 10.7 g (38%) of the title compound as a white foam: IR (KBr) 3430, 1744, 1720, 1270, 1248, 972 cm^{-1}. Diol 9 was also obtained in 17% yield.

3α-*O*-(2-Tetrahydropyranyl)scirpene-4β,15-diol (9). To a solution of 15 (1.07 g, 2.37 mmol) in 40 mL of THF and MeOH (5:3) was added 40 mL of 0.3 N NaOH solution. After 20 h of stirring at room temperature, the resulting solution was diluted with CH_2Cl_2 and washed with brine. Drying over K_2CO_3 and removal of the solvent gave 891 mg of foam, which was subsequently chromatographed on silica gel. Elution with 1% MeOH–CH_2Cl_2 gave 46 mg (5%) of 8. A further elution with 5% MeOH–CH_2Cl_2 gave 808 mg (93%) of the title compound as an amorphous solid: IR (KBr) 3457, 1445, 1135, 1020, 978 cm^{-1}.

15-Acetoxy-4β-(chloroacetoxy)scirpen-3α-ol (10). A mixture of 8 (2.88 g, 7.0 mmol), chloroacetic anhydride (2.0 g, 10.5 mmol), and pyridine (2.80 g, 17.5 mmol) in 100 mL of CH_2Cl_2 was stirred at room temperature for 64 h. After a usual workup, 3.34 g of foam was obtained. This material was chromatographed on silica gel (elution 1% MeOH–CH_2Cl_2) to give 2.80 g (83%) of 15-acetoxy-4β-(chloroacetoxy)-3α-*O*-(2-tetrahydropyranyl)scirpene, which was characterized by its IR and NMR spectra.

This material was dissolved in 150 mL of 95% EtOH and treated with 25 mL of 10% HCl solution at room temperature for 40 h. The solvent was evaporated under reduced pressure and the residue was recrystallized from Et_2O to give 1.96 g (80%) of the title compound: mp 166–168 °C; IR (KBr) 3500, 1754, 1736, 1378, 1260, 1074 cm^{-1}. Anal. ($C_{19}H_{25}ClO_7$) C, H.

4β,15-Bis(chloroacetoxy)-3α-*O*-(2-tetrahydropyranyl)-scirpene (16). A mixture of 9 (808 mg, 2.21 mmol), chloroacetic anhydride (1.13 g, 6.62 mmol), and pyridine (894 mg, 11.1 mmol) in 100 mL of CH_2Cl_2 was stirred at room temperature for 14 h. After the usual workup, the residue was chrmatographed on silica gel (elution with 0.5% MeOH–CH_2Cl_2) to give 1.06 g (92%) of the title compound as a white foam: IR (KBr) 1762, 1740, 1290, 1172, 1080 cm^{-1}.

4β,15-Bis(chloroacetoxy)scirpen-3α-ol (11). To a solution of 16 (858 mg, 1.65 mmol) in 100 mL of 95% EtOH was added 19 mL of 1 N HCl solution. The resulting solution was stirred at room temperature for 24 h. After a usual workup, the residue was chromatographed on silica gel (elution with 1% MeOH–CH_2Cl_2) to give 524 mg (73%) of 11. An analytical sample was obtained by recrystallization from $CHCl_3$–Et_2O: mp 139–141 °C; IR (KBr) 3450, 1758, 1742, 1327, 1293, 1173 cm^{-1}. Anal. ($C_{19}H_{24}Cl_2O_7$) C, H.

4β,15-Bis(bromoacetoxy)scirpen-3α-ol (12). This compound was obtained in an analogous manner to 11, with the exception that 2,6-lutidine was used as base in the reaction of 9 with bromoacetyl bromide: yield 53%; mp 125–126 °C (Et_2O); IR (KBr) 3480, 1750, 1735, 1280, 1165, cm^{-1}. Anal. ($C_{19}H_{24}Br_2O_7$) H; C: calcd, 43.53; found, 45.47.

15-(Crotonyloxy)scirpene-3α,4β-diol (17). To a solution of 9 (366 mg, 1 mmol) and 395 mg (5 mmol) of pyridine in 50 mL of CH_2Cl_2 was added 261 mg (2.5 mmol) of crotonyl chloride at 5 °C. After 16 h of stirring at room temperature, the solution was diluted with 50 mL of CH_2Cl_2 and washed with saturated $NaHCO_3$ solution and brine. Drying over Na_2SO_4 and removal of the solvent gave 360 mg of oil. This material was dissolved in 50 mL of 95% EtOH and treated with 5 mL of 2 N HCl solution at room temperature for 22 h. After a usual workup, the residue was chromatographed on silica gel (elution with 1% MeOH–CH_2Cl_2) to give 26 mg of 13, 22 mg of 18, and 147 mg (42%) of the title compound: mp 83–83 °C ($CHCl_3$–petroleum ether); IR (KBr) 3440, 1725, 1190, 1085, 965 cm^{-1}. Anal. ($C_{19}H_{26}O_6 \cdot 0.5H_2O$) C, H.

4β,15-Bis(crotonyloxy)scirpen-3α-ol (13) and 4β-(Crotonyloxy)scirpene-3α,15-diol (18). The title compounds were prepared in the same manner as described for 17, except that 6 molar equiv of crotonyl chloride was used. Chromatography on silica gel (elution with 1% MeOH–CH_2Cl_2) gave 13 as a white foam: yield 10%; IR (KBr) 3420, 1720, 1310, 1260, 965 cm^{-1}. A further elution provided 18 as an amorphous solid: yield 19%; mp 60–62 °C (CH_2Cl_2–petroleum ether); IR (KBr) 3460, 1710, 1315, 1190, 1080 cm^{-1}. Anal. ($C_{19}H_{26}O_6 \cdot 0.5H_2O$) C, H.

4β,15-Bis(methacryloyloxy)scirpen-3α-ol (14) and 4β-(Methacryloyloxy)scirpene-3α,15-diol (20). The title compounds were prepared in the same manner as for 17, except that 5 molar equiv of methacryloyl chloride was used. A silica gel chromatography (elution with 1% MeOH–CH_2Cl_2) gave 14 as an amorphous solid: yield 5%; IR (KBr) 3500, 1720, 1165, 1080, 960 cm^{-1}. Subsequent elution gave 20 as colorless crystals: yield 3%; mp 175–176 °C (Et_2O); IR (KBr) 3510, 1690, 1330, 1170, 900 cm^{-1}. Anal. ($C_{19}H_{26}O_6 \cdot 0.25H_2O$) C, H. Compound 19 was also obtained in 5% yield.

15-(Methacryloyloxy)scirpene-3α,4β-diol (19). Using 2.5 molar equiv of methacryloyl chloride as an acylating agent, we prepared the title compound in the same manner as described for 17: yield 33%; mp 79–81 °C (CH_2Cl_2–petroleum ether); IR (KBr) 3440, 1715, 1165, 1080, 955 cm^{-1}. Anal. ($C_{19}H_{26}O_6 \cdot 0.5H_2O$) C, H.

4β-(Chloroacetoxy)-15-(methacryloyloxy)scirpen-3α-ol (21). Chloroacetic anhydride (78 mg, 0.46 mmol) was added to a CH_2Cl_2 (5 mL) solution of 15-(methacryloyloxy)-3α-*O*-(2-tetrahydropyranyl)scirpen-4β-ol and 36 mg of (0.46 mmol) of pyridine. After 17 h of stirring at room temperature, the solution was worked up in the usual manner. Hydrolysis of the THP group

as before furnished 155 mg (96%) of the title compound: IR (KBr) 755, 1715, 1320, 1295, 1085 cm^{-1}. Anal. ($C_{21}H_{27}ClO_7$) H; C: calcd, 59.08; found, 60.48.

4β,15-Diacetoxyscirpen-3-one (22). Trifluoroacetic anhydride (861 mg, 6.83 mmol) was added dropwise at −78 °C to a solution of Me_2SO (534 mg, 6.83 mol) in 15 mL of dry CH_2Cl_2. After the mixture was stirred 10 min, a solution of anguidin (1.00 g, 2.73 mmol) in 10 mL of CH_2Cl_2 was added, and stirring was continued at −78 °C for 30 min. Triethylamine (691 mg, 6.83 mmol) was added, and the resulting solution was warmed to room temperature. The solution was diluted with CH_2Cl_2 and washed with brine. Drying over Na_2SO_4 and removal of the solvent gave 975 mg (98%) of solid. Recrystallization from ether furnished the analytical sample: mp 160–161 °C (lit.[7] mp 161–162 °C). The following ketones were prepared similarly from the alcohols indicated.

15-Acetoxy-4β-(chloroacetoxy)scirpen-3-one (26) from 10: yield 86%; mp 151–152.5 °C (Et_2O–hexane); IR (KBr) 1776, 1734, 1280, 1258, 1052 cm^{-1}. Anal. ($C_{19}H_{23}ClO_7$) C, H; Cl: calcd, 8.89; found, 8.47.

4β,15-Bis(chloroacetoxy)scirpen-3-one (27) from 11: yield 93%; IR (KBr) 1760, 1745, 1316, 1340, 1165, 1008 cm^{-1}.

4β,15-Bis(methacryloyloxy)scirpen-3-one (28) from 14: yield 95%; IR (KBr) 1770, 2725, 1160, 1060, 960 cm^{-1}.

4β-(Chloroacetoxy)-15-(methacryloyloxy)scirpen-3-one (29) from 21: yield 97%; IR (KBr) 1775, 1720, 1300, 1165, 1065 cm^{-1}.

3α,15-Bis(chloroacetoxy)scirpen-4-one (30) from 6: yield 23%; mp 99–100 °C; IR (KBr) 1760, 1740, 1285, 1160, 950 cm^{-1}; NMR ($CDCl_3$) δ 1.00 (s, 3 H), 1.78 (s, 3 H), 1.2–2.2 (m, 4 H), 3.01 (d, 1 H, J = 4 Hz), 3.28 (d, 1 H, J = 4 Hz), 3.80 (d, 1 H, J = 5 Hz), 4.09 (s, 2 H), 4.12 (s, 2 H), 4.28 (s, 2 H), 4.1 5–4.3 (m, 1 H), 5.44 (d, 2 H, J = 5 Hz). Anal. ($C_{19}H_{22}Cl_2O_7$) C, H, Cl.

Oxime of 4β,15-Diacetoxyscirpen-3-one (23) and the Oxime of 15-Acetoxy-4β-hydroxyscirpen-3-one (24). To a solution of **22** (364 mg, 1.0 mmol) in 60 mL of MeOH was added a solution of NH_2OH·HCl (336 mg, 4.57 mmol) and NaOAc (366 mg, 2.47 mmol) in 7 mL of water. After 15 h of stirring at room temperature, the solution was worked up in the usual manner. A silica gel chromatography (elution with 2% MeOH–CH_2Cl_2) of the resulting material gave 185 mg (49%) of **23** as an amorphous solid. The NMR indicated it was an approximately 2:1 mixture of *syn*- and *anti*-oximes: IR (KBr) 1741, 1720, 1673, 1370, 1249, 1032 cm^{-1}. The second component (49 mg, 15%) that was eluted with 3% MeOH–CH_2Cl_2 was characterized to be an approximately 3:1 mixture of *syn*- and *anti*-oximes **24**: IR (KBr) 3410, 1741, 1716, 1675, 1242, 1047 cm^{-1}.

***p*-Toluenesulfonylhydrazone of 4β,15-Diacetoxyscirpen-3-one (25).** A mixture of **22** (304 mg, 1 mmol) and *p*-toluenesulfonylhydrazide (205 mg, 1.1 mmol) in 4 mL of THF and 5 mL of EtOH was stirred at room temperature for 66 h. Removal of the solvent gave a white foam, which was shown by NMR to be a mixture of *syn*- and *anti*-**25**. Chromatography on silica gel (elution with 0.5% MeOH–CH_2Cl_2) gave 92 mg (17%) of the pure anti isomer. A further elution with 0.5% MeOH–CH_2Cl_2 gave 311 mg (59%) of a mixture of syn and anti isomers. The anti isomer was recrystallized from ether: mp 100–103 °C; IR (KBr) 1745, 1722, 1680, 1372, 1250 cm^{-1}.

4β,15-Diacetoxyverrucarol (31) and 15-Acetoxyverrucarol (32). Verrucarol was obtained by hydrolysis of verrucarin A as described.[16] Verrucarol (54 mg, 0.21 mmol) was treated with 0.25 mL of acetic anhydride and 0.25 mL of pyridine at room temperature for 2 h. The residue obtained after removal of the excess reagents was chromatographed on silica gel (elution with 0.5% MeOH–CH_2Cl_2) to give 26 mg (37%) of **31**: IR (KBr) 1735, 1262, 1250, 1080 cm^{-1}; NMR ($CDCl_3$) δ 0.79 (s, 3 H), 1.70 (s, 3 H), 1.77–2.40 (m, 11 H), 2.52 (2 d, 1 H, J = 8 and 15 Hz), 2.80 (d, 1 H, J = 4 Hz), 3.10 (d, 1 H, J = 4 Hz), 3.74 (d, 1 H, J = 5 Hz), 3.81 (d, 1 H, J = 5 Hz), 4.02 (d, 1 H, J = 12 Hz), 4.10 (d, 1 H, J = 12 Hz), 5.43 (d, 1 H, J = 5 Hz), 5.75 (2 d, 1 H, J = 4 and 8 Hz).

On further elution with the same solvent, 8 mg (13%) of **32** was obtained: IR (KBr) 3440, 1720, 1251, 1070, 958 cm^{-1}; NMR ($CDCl_3$) δ 0.79 (s, 3 H), 1.74 (s, 3 H), 1.8–2.2 (m, 8 H), 2.61 (2 d, 1 H, J = 8 and 16 Hz), 2.82 (d, 1 H, J = 4 Hz), 3.14 (d, 1 H, J = 4 Hz), 3.62 (d, 1 H, J = 6 Hz), 3.84 (d, 1 H, J = 8 Hz), 3.93 (d, 1 H, J = 12 Hz), 4.17 (d, 1 H, J = 12 Hz), 4.50 (m, 1 H), 5.42 (d, 1 H, J = 5 Hz).

15-Hydroxyscirpen-3-one (33). This compound was prepared from 15-acetoxy-3α,4β-bis(mesyloxy)scirpene according to the published method:[7] yield 30%; mp 168–170 °C (CH_2Cl_2–Et_2O) (lit.[7] mp 171–175 °C).

15-Acetoxyscirpen-3-one (34). This compound was prepared by acetylation of **33** as described:[7] yield 70%; mp 159–160 °C (Et_2O–hexane) (lit.[7] mp 154–162 °C).

15-Acetoxy-3α-(mesyloxy)scirpen-4-one (35). The title compound was prepared by the literature method:[7] yield 70%; mp 187–188 °C (acetone–hexane) (lit.[7] mp 186–190 °C).

4β,15-Diacetoxy-9β,10β-epoxyscirpen-3α-ol (36). A solution of **1** (100 mg, 0.27 mmol) and *m*-chloroperoxybenzoic acid (54 mg, 0.32 mmol) in 50 mL of CH_2Cl_2 was stirred at room temperature for 72 h. The resulting solution was washed with 20% Na_2SO_3 solution and 2 N $NaHCO_3$ solution. Drying over Na_2SO_4 and removal of the solvent gave 112 mg of oil. Preparative TLC on silica gel developed with CH_2Cl_2–EtOAc (2:1) and precipitation of the chromatographed material gave 75 mg (73%) of **36** as an amorphous solid: mp 77–80 °C; IR (KBr) 1743, 1728, 1323, 1250, 1060 cm^{-1}. The following epoxides were prepared in the same manner as **36**.

4β,15-Bis(crotonyloxy)-9β,10β-epoxyscirpen-3α-ol (37) from 13: yield 87%; mp 68–70 °C (Et_2O–petroleum ether); IR (KBr) 3460, 1720, 1310, 1255, 1175 cm^{-1}. Anal. ($C_{23}H_{30}O_8$·0.5H_2O) H; C: calcd, 63.58; found, 62.14.

15-(Crotonyloxy)-9β,10β-epoxyscirpene-3α,4β-diol (38) from 17: yield 27%; mp 83–85 °C (Et_2O–petroeum ether); IR (KBr) 3440, 1710, 1180, 1070, 965 cm^{-1}. Anal. ($C_{19}H_{26}O_7$·0.5H_2O) C, H.

4β,15-Diacetoxy-9β,10β-epoxyscirpen-3-one (39). In a manner similar to that described for **22**, the title compound was prepared in 93% yield starting with **36**: mp 219–110 °C (CH_2Cl_2–Et_2O); IR (KBr) 1775, 1749, 1377, 1283, 1260, 1081, cm^{-1}. Anal. ($C_{19}H_{24}O_8$) H; C: calcd, 59.99; found, 59.46.

4β-Hydroxy-3α-*O*-(2-tetrahydropyranyl)scirpene-15-carboxaldehyde (40). A solution of **9** (732 mg, 2.0 mmol), pyridinium chlorochromate (647 mg, 3.0 mmol), and sodium acetate (164 mg, 2.0 mmol) in 10 mL of CH_2Cl_2 was stirred at room temperature for 1 h. After the usual workup, the residue was chromatographed on silica gel (elution with 0.75% MeOH–CH_2Cl_2) to give 523 mg (72%) of **40** as a mixture of diastereomers. From fractions rich in one diastereomer, one isomer was crystallized from CH_2Cl_2–Et_2O: mp 163–165 °C; IR (KBr) 3460, 2940, 1720, 1120, 1075, 972 cm^{-1}.

3α,4β-Dihydroxyscirpene-15-carboxaldehyde (41). The THP ether (**40**; 200 mg, 0.62 mmol) was dissolved in 80 mL of 95% EtOH and was treated with 16 mL of 1 N HCl for 4 h at room temperature. The usual workup gave 118 mg (77%) of **41** as a colorless solid: mp 131–133 °C (Et_2O); IR (KBr) 3420, 1717, 1165, 868, 710 cm^{-1}.

4β-Acetoxy-3α-*O*-(2-tetrahydropyranyl)scirpene-15-carboxaldehyde (42). A diastereomeric mixture of **40** (1.90 g, 5.22 mmol) was dissolved in 3.0 mL of pyridine and was treated with 3.0 mL (31.8 mmol) of acetic anhydride at room temperature for 3.5 h. After the usual workup, the residue was chromatographed on silica gel (elution with 0.5% MeOH–CH_2Cl_2). The purified material was crystallized from Et_2O to give 1.85 g (77%) of a colorless solid; mp 128–130 °C; IR (KBr) 2950, 1745, 1715, 1235, 1125, 1035 cm^{-1}.

4β-Acetoxy-3α-hydroxyscirpene-15-carboxaldehyde (43) from 42. This compound was obtained in the same manner described for **41**: yield 45%; mp 122–124 °C (Et_2O); IR (KBr) 3500, 1740, 1720, 1225, 1075, 960 cm^{-1}. Anal. ($C_{17}H_{22}O_6$) C, H.

Semicarbazone of 4β-Acetoxy-3-*O*-(2-tetrahydropyranyl)scirpene-15-carboxaldehyde (44). Semicarbazide hydrochloride (600 mg, 5.38 mmol) and 900 mg of sodium acetate were added to a solution of **42** (250 mg, 0.62 mmol) in 6 mL of EtOH containing a small amount of water. The mixture was stirred at room temperature for 24 h. After a usual workup, the residue was chromatographed on silica gel (elution with 2% MeOH–CH_2Cl_2) to give 221 mg (78%) of crystalline **44**: mp 126–128 °C (CH_2Cl_2–Et_2O); IR (KBr) 3490, 1735, 1695, 1570, 1240, 1035 cm^{-1}. Anal. ($C_{23}H_{33}N_3O_7$) C, H, N.

Semicarbazone of 4β-Acetoxy-3α-hydroxyscirpene-15-carboxaldehyde (45). The THP group of 44 was cleaved in the same manner described for 41. After the usual workup, the resulting solid was recrystallized from CH_2Cl_2–Et_2O: yield 71%; mp 202–204 °C; IR (KBr) 3480, 3380, 1725, 1685, 1580, 1245, 1075, 960 cm^{-1}. Anal. ($C_{18}H_{25}N_3O_6$) H; C: calcd, 56.27; found, 57.05; N: calcd 11.08; found, 10.52.

3α,4β-Dihydroxyscirpene-15-carboxylic Acid (46). An aqueous $KMnO_4$ solution (0.1 M, 60 mL) was added to a solution of 40 (2.0 g, 5.49 mmol) in 120 mL of acetone. After 1 h of stirring at room temperature, the solution was worked up in the usual manner. The residue was dissolved in CH_2Cl_2 and extracted with saturated aqueous $NaHCO_3$ solution. The aqueous phase was then acidified with 42% H_3PO_4 and extracted with EtOAc. The residue (450 mg) obtained after evaporation of EtOAc was dissolved in 54 mL of EtOH and was treated with 11 mL of 1 N HCl solution at room temperature for 18 h. Extraction of the aqueous layer with EtOAc and evaporation of the solvent gave 210 mg (14%) of 46: mp 192–193 °C ($CHCl_3$); IR (KBr) 3450, 3240, 2900, 2600, 1690, 1190, 1050, 955 cm^{-1}. Anal. ($C_{15}H_{20}O_6 \cdot 0.5H_2O$) C, H.

4β,15-Diacetoxyscirpene-3α,8β-diol (48). A mixture of 1 (366 mg, 1.0 mmol) and SeO_2 (122 mg, 1.1 mmol) in 25 mL of dioxane containing 1 mL of water was heated to reflux for 24 h. The resulting solution was filtered through Celite, and the residue was washed with a small amount of CH_2Cl_2. The combined filtrate was diluted with CH_2Cl_2 and washed with brine. Drying over Na_2SO_4 and removal of the solvent gave a yellow oil. Chromatography on silica gel (elution with 2% MeOH–CH_2Cl_2), followed by crystallization from CH_2Cl_2 and Et_2O, gave 148 mg (39%) of slightly pink crystals: mp 114–116 °C; IR (KBr) 3435, 1730, 1715, 1365, 1248, 1080 cm^{-1}. Anal. ($C_{19}H_{26}O_8$) H; C: calcd, 59.67; found, 59.23.

15-Acetoxy-4β-(chloroacetoxy)scirpene-3α,8β-diol (49). This compound was prepared in the same manner as 48 from 10: yield 42%; mp 199.5–200.5 °C (Et_2O); IR (KBr) 3505, 1740, 1730, 1255, 1165 cm^{-1}. Anal. ($C_{19}H_{25}ClO_8$) C, H.

4β,15-Diacetoxy-3α-hydroxyscirpen-8-one (50). A mixture of 48 (270 mg, 0.71 mmol), pyridinium chlorochromate (22.3 mg, 1.03 mmol), and anhydrous NaOAc (29 mg, 0.35 mmol) in 15 mL of CH_2Cl_2 was stirred at room temperature for 2.5 h. After the mixture was filtered through Celite, the solvent was removed under reduced pressure to give 360 mg of oil. Chromatography on silica gel (elution with 0.75% MeOH–CH_2Cl_2) gave 218 mg (81%) of the title compound as a white foam: IR (KBr) 3445, 1740, 1678, 1366, 1240, 1042 cm^{-1}. Anal. ($C_{19}H_{24}O_8$) H; C: calcd, 59.99; found, 59.36.

4β,15-Diacetoxyscirpene-3α,8α-diol (51). To a solution of 50 (261 mg, 0.69 mmol) in 25 mL of THF was added 1.52 mL of DIBAH (1 M solution in hexane) at −78 °C. After 4 h of stirring at −78 °C, the reaction was worked up in the usual manner. The residue was chromatographed on silica gel (elution with 1.5% MeOH–CH_2Cl_2) to give 89 mg (38%) of the title compound: mp 167–168 °C (CH_2Cl_2–Et_2O) (lit.[21] mp 171–172 °C); IR (KBr) 3440, 1730, 1260, 1050 cm^{-1}. Anal. ($C_{19}H_{26}O_8$) H; C: calcd, 59.67; found, 59.26.

On further elution, 48 mg (18%) of 48 was also isolated. Compound 51 was also prepared from a mixture of 52 and 53 by the following method.

A mixture of 52 and 53 (8.90 g, 20 mmol) was dissolved in 200 mL of THF and 100 mL of H_2O. A 5% $NaHCO_3$ solution (84 mL) and silver trifluoroacetate (2.21 g, 10 mmol) were added. After 48 h of stirring at room temperature in the dark, the solution was filtered through Celite. Removal of the THF and extraction of the aqueous residue with CH_2Cl_2 gave 8.0 g of oil. This oil was chromatographed on silica gel (flash chromatography,[27] elution with 25% EtOAc in CH_2Cl_2) to give 1.10 g (14%) of the title compound. Epimer 48 was also isolated in 33% yield.

4β,15-Diacetoxy-8-bromoscirpen-3α-ol (52 and 53). A solution of 1 (1.10 g, 3.0 mmol) and *N*-bromosuccinimide (587 mg, 3.3 mmol) in 40 mL of CH_2Cl_2 was irradiated with an incandescent lamp for 10 min. The resulting solution was washed with brine and dried over Na_2SO_4. Removal of the solvent gave 1.44 g of white foam, which was chromatographed on silica gel (elution with Et_2O–heptane, 3:2). The first fraction was identified by NMR as 53: yield 573 mg (43%); NMR ($CDCl_3$) δ 0.76 (s, 3 H), 1.89 (s, 3 H), 2.01 (s, 3 H), 2.09 (s, 3 H), 2.36 (3 d, 1 H, J = 2, 6, and 12 Hz), 2.66 (2 d, 1 H, J = 11 and 12 Hz), 2.77 (d, 1 H, J = 4 Hz), 3.06 (d, 1 H, J = 4 Hz), 3.17 (br s, 1 H), 3.66 (d, 1 H, J = 5 Hz), 3.90 (d, 1 H, J = 13 Hz), 3.99–4.22 (m, 2 H), 4.18 (d, 1 H, J = 13 Hz), 4.53 (2 d, 1 H, J = 6 and 11 Hz), 5.02 (d, 1 H, J = 3 Hz), 5.68 (d, q, 1 H, J = 1 and 5 Hz). The second fraction afforded 102 mg (8%) of 52: mp 171–173 °C (CH_2Cl_2–Et_2O); NMR ($CDCl_3$) δ 0.94 (s, 3 H), 1.99 (s, 3 H) 2.14 (s, 3 H), 2.22 (s, 3 H), 2.42 (d, t, 1 H, J = 1 and 16 Hz), 2.87 (2 d, 1 H, J = 6 and 16 Hz), 2.82 (d, 1 H, J = 4 Hz), 3.12 (d, 1 H, J = 4 Hz), 3.75 (d, 1 H, J = 5 Hz), 4.20 (2 d, 1 H, J = 3 and 5 Hz), 4.24 (d, 1 H, J = 12 Hz), 4.36 (d, 1 H, J = 5 Hz), 4.40 (d, 1 H, J = 12 Hz), 4.82 (2 d, 1 H, J = 1 and 6 Hz), 5.25 (d, 1 H, J = 3 Hz), 5.89 (d, 1 H, J = 5 Hz); IR (KBr) 3420, 1737, 1360, 1245, 1045, 969 cm^{-1}. Anal. ($C_{19}H_{25}BrO_7$) C, H.

4β,15-Diacetoxyscirpene-3,8-dione (54). To a solution of 78 mg (1.0 mmol) of Me_2SO in 2 mL of CH_2Cl_2 was added a 10% CH_2Cl_2 solution of trifluoroacetic anhydride (0.6 mmol) at −78 °C. After the mixture was stirred for 10 min at −78 °C, a solution of 48 (76 mg, 0.2 mmol) in 2 mL of CH_2Cl_2 was added dropwise. Stirring was continued at the same temperature for 30 min, and then triethylamine (101 mg, 1 mmol) was added. After an additional 10 min of stirring, the reaction mixture was warmed to room temperature. It was diluted with CH_2Cl_2 and washed with brine. Drying over Na_2SO_4 and removal of the solvent gave 58 mg (76%) of crystalline solid. Recrystallization from CH_2Cl_2–Et_2O provided the analytical sample: mp 198–199 °C; IR (KBr) 1780, 1676, 1392, 1370, 1231, 1220, 1065, 1035 cm^{-1}. Anal. ($C_{19}H_{22}O_8$) C, H.

15-Acetoxy-4β-(chloroacetoxy)scirpene-3,8-dione (55). This compound was prepared in 66% yield from 49 using the procedure similar to that described for 54: mp 169.5–171 °C (Et_2O); IR (KBr) 1772, 1742, 1678, 1232, 1219, 1156, 1052 cm^{-1}. Anal. ($C_{19}H_{21}ClO_8$) H; C: calcd, 55.28; found, 54.31.

3α,8β-Bis-*O*-(2-tetrahydropyranyl)scirpene-4β,15-diol (56). A solution of 48 (5.0 g, 13 mmol) in 220 mL of CH_2Cl_2 containing 2.75 g (33 mmol) of dihydropyran and 85 mg (0.33 mmol) of pyridinium tosylate[28] was stirred at room temperature for 24 h. After the usual workup, the residue was chromatographed on silica gel (elution with 10% EtOAc–CH_2Cl_2) to give 6.97 g (97%) of 4α,15-diacetoxy-3α,8β-bis-*O*-(2-tetrahydropyranyl)scirpene.

This material (6.90 g, 12.5 mmol) was dissolved in 175 mL of MeOH, and after the addition of 20 mL of 10% NaOH solution, the mixture was kept at room temperature for 40 min. The resulting solution was partitioned between CH_2Cl_2 and water, and the aqueous layer was extracted with fresh CH_2Cl_2. The combined CH_2Cl_2 layers were dried over Na_2SO_4. Removal of the solvent gave 5.81 g (99%) of the title compound: IR (KBr) 3470, 1600, 1160, 1075, 1033, 975 cm^{-1}; NMR ($CDCl_3$) δ 0.94 (d, 3 H), 1.39–2.38 (m, 17 H), 2.80 (m, 1 H), 3.06 (m, 1 H), 3.37–4.50 (m, 11 H), 4.62–4.98 (m, 2 H), 5.56 (m, 1 H).

15-Acetoxy-4β-(methacryloyloxy)-3α,8β-bis-*O*-(2-tetrahydropyranyl)scirpene (57). A solution of acetyl chloride (724 mg, 9.22 mmol) in 10 mL of CH_2Cl_2 was added at 0 °C to a solution of 56 (4.30 g, 9.22 mmol) and triethylamine (930 mg, 9.22 mmol) in 200 mL of CH_2Cl_2. After 17 h of stirring at 0 °C, the usual workup gave 4.58 g (98%) of 15-acetoxy-3α,8β-bis-*O*-(2-tetrahydropyranyl)scirpen-4β-ol.

This material (2.03 g, 4.0 mmol) was dissolved in 35 mL of CH_2Cl_2. To this solution was added triethylamine (1.01 g, 10 mmol) and 15 mL of a CH_2Cl_2 solution of methacryloyl chloride (1.04 g, 10 mmol). The usual workup after 12 h of stirring at room temperature gave 1.95 g (88%) of 57 as a white foam: NMR ($CDCl_3$) δ 0.78 (s, 3 H), 1.40–2.22 (m, 23 H), 2.82 (t, 1 H, J = 3 Hz), 3.32–3.63 (m, 2 H), 3.70–4.46 (m, 5 H), 4.54–4.88 (m, 2 H), 5.47–5.88 (m, 2 H), 5.84 (m, 1 H), 6.24 (m, 1 H).

15-Acetoxy-4β-(methacryloyloxy)scirpene-3α,8β-diol (58). A solution of 57 (1.95 g, 3.4 mmol) and pyridinium tosylate (60 mg, 0.3 mmol) in 60 mL of 95% EtOH was stirred at 50 °C for 24 h. The residue obtained after removal of EtOH was dissolved

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in CH_2Cl_2 and washed with water. Drying over Na_2SO_4 and removal of the solvent gave 750 mg (54%) of **58**: mp 164–165 °C (EtOAc–petroleum ether); IR (KBr) 1745, 1725, 1250, 1170, 1075, 965 cm^{-1}. Anal. ($C_{21}H_{28}O_8$) C, H.

15-Acetoxy-4β-(methacryloyloxy)scirpene-3,8-dione (59). This compound was prepared in 78% yield from **58** using the procedure similar to that described for **54**: mp 165–166 °C (Et_2O–petroleum ether); IR (KBr) 1775, 1750, 1728, 1680, 1236, 1150, 1066 cm^{-1}. Anal. ($C_{21}H_{24}O_8$) C, H.

4β,15-Bis(methacryloyloxy)scirpene-3α,8β-diol (60). A solution of **56** (1.24 g, 2.6 mmol), methacryloyl chloride (1.36 g, 31 mmol), and triethylamine (1.01 g, 10 mmol) in 50 mL of CH_2Cl_2 was stirred at room temperature for 24 h. The usual workup gave 1.20 g (76%) of 4α,15-bis(methacryloyloxy)-3α,8β-bis-*O*-(2-tetrahydropyranyl)scirpene. Treatment of this material with pyridinium tosylate as described for **58** gave 595 mg (76%) of the title compound: mp 143–144 °C (Et_2O); IR (KBr) 3520, 3280, 1715, 1695, 1300, 1165, 965 cm^{-1}. Anal. ($C_{23}H_{30}O_8$) C; H: calcd, 6.96; found, 7.66.

4β,15-Bis(methacryloyloxy)scirpene-3,8-dione (61). This compound was prepared in 77% yield from **60** using the procedure similar to that described for **54**: mp 132–135 °C (Et_2O–petroleum ether): IR (KBr) 1772, 1725, 1685, 1150, 1060, 948 cm^{-1}. Anal. ($C_{23}H_{26}O_8 \cdot 0.125H_2O$) H; C: calcd, 64.17; found, 63.76.

Biological Testing. Antitumor Effects. The tumors and parameters used in evaluating the analogues are summarized in Table III. The analogues were dissolved in Me_2SO, and futher dilutions were made with saline. All drug injections were made intraperitoneally. Tests with P-388 and L1210 leukemias, B16 melanoma, and Lewis lung carcinoma were conducted as described before.[22] The percent T/C is defined as the median survival time (MST) of all mice in a drug-treated (T) group divided by MST of the tumor control (C) group × 100.

In the Colon 38 test, mice were treated daily for 5 days beginning on day 15 and again on day 26 post-implant. Mice bearing Colon 36 were treated daily for 5 days beginning on day 3 and again on day 14 post-implant. In these two tests, the antiumor activity was judged on (a) the relative median time for tumors to reach a predetermined size (e.g., 750 mg for Colon 36, and 1250 mg for Colon 38) in drug-treated (T) as compared to control (C) mice (i.e., T – C). These tests were conducted by Dr. T. H. Corbett of Southern Research Institute, Birmingham, AL.

Effects on Protein Synthesis in H-HeLa Cells. All procedures were carried out as described previously.[6] When used, H-HeLa cells were growing exponentially with a density of 4×10^5 cells/mL. Cultures of these cells were transferred to a medium lacking amino acids and serum, and were incubated at 37 °C for 15 min before administration of the test analogue. Then, L-[4,5-^{3}H]leucine (1 Ci/mmol) was added, together with a trichothecene analogue (time zero), and incubation was continued at 37 °C. The analogues were dissolved in Me_2SO so that the final concentration of Me_2SO in reaction mixtures never exceeded 1% (v/v). Samples of 1 mL each were taken at various times into 1 mL of 10% (w/v) trichloroacetic acid, held at 90 °C for 20 min, and then cooled in ice. Precipitated material was collected on Whatman GF/C glass-fiber disks, which were washed three times with 5% trichloroacetic acid, dried, and prepared for liquid scintillation counting according to standard procedures.[26]

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Notes

Synthesis and Some Pharmacological Properties of Z-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp(Phe-NH_2)-OH, a 32-β-Aspartyl Analogue of Cholecystokinin (Pancreozymin) 27–33

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The heptapeptide carbobenzoxy-L-tyrosyl(*O*-sulfate)-L-methionylglycyl-L-tryptophyl-L-methionyl-L-aspartyl-β-L-phenylalanine amine (Z-32-β-Asp-CCK–27–33) was synthesized and tested for its ability to stimulate amylase secretion from dispersed pancreatic acini in vitro, to increase protein secretion from cat pancreas in vivo, and to cause contraction of guinea pig gallbladder in situ. In increasing amylase secretion in vitro, the Z-32-β-Asp-CCK–27–33 was equal in efficacy with but approximatively one-third as potent as the Boc-CCK–27–33, and when tested in vivo its activity is approximately 10 Ivy dog units (Idu)/μg. In stimulation of the contraction of the gallbladder, it showed an activity lower than 1 Idu/μg. This analogue has more pancreozyminic activity than cholecystokin-like activity. This seems to indicate different affinities for the two receptors.

In an earlier study[1] on the synthesis and properties of the desamino derivative of the C-terminal heptapeptide segment of cholecystokinin (desamino-CCK–27–33), formation of a byproduct was observed.[2] It occurred during the preparation of the sulfate ester of the phenolic hydroxyl group of the N-terminal tyrosine residue. The properties and the conditions of the formation of this by

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