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Antitumor Activity of Some Microbial and Chemical Transformation Products of Anguidine (4,15-Diacetoxyscirpene-3-ol)

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Summary. *The in vivo antitumor activities, as measured by inhibition of transplanted P-388 and L-1210 leukemia in mice, have been determined for a series of analogs of anguidine including triacetoxyscirpenol, the three diacetoxyscirpenols, the three monacetoxyscirpenols, and scirpenetriol. An acetoxy function at position 15 appears to be required for good activity.*

Introduction

We have recently reported that anguidine (12,13-epoxytrichothec-9-ene 4β,15-diacetoxy-3α-ol, or 4,15-diacetoxyscirpenol) can be transformed to a number of closely related derivatives through a combination of microbial and chemical modifications [2, 3]. The organsims that carry out the transformations are *Mucor mucedo* (ATCC 7,941), *Streptomyces griseus* (NRRL 3,242), *Acinetobacter calcoaceticus* (A 22,234), and *Fusarium oxysporum* f. sp. *vasinfectum* (ATCC 7,808). Activities of these derivatives in antifungal and tissue culture cytotoxicity tests have already been reported [2, 3]. We now wish to report on the activities of these compounds in animal tumor systems.

Anguidine is known to have significant antitumor activity in experimental systems [4]. Phase I clinical trials of this compound have been completed in the United States [6, 7], and Phase II clinical trials are under way. We have previously reported [4] that one of the anguidine analogs that could be produced either by chemical modification or reaction with *Acinetobacter* cells, namely 15-acetoxyscirpene-3,4-diol, has significantly greater potency than anguidine when tested in an in vivo P-388 lymphatic leukemia test in mice. This same derivative, however, did not appear to be as active as the parent anguidine when compared in antifungal or tissue culture cytotoxicity tests [2].

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This paper reports the results of the measurement of the comparative in vivo antitumor activities of a number of mono-, di-, and triacetoxy derivatives of scirpenetriol, which we have obtained in our transformation studies.

Materials and Methods

The compounds reported here were produced by either microbial or chemical modification of anguidine, as outlined previously [2, 3]. The structures of these compounds are given in Fig. 1.

In vivo tumor tests on P-388 and L-1210 leukemia were performed according to previously published procedures [1, 5]. In each case, tumor cells obtained from DBA/2 passage mice were inoculated IP to sets of CDF_1 or BDF_1 hybrid mice (10^6 cells/mouse) for therapeutic experiments. Treatment was always started 24 h after tumor implantation and continued once daily for 9 days by the IP route, with a constant volume of 0.5 ml/injection. Evaluation was on the basis of median survival time (MST). The effect, T/C = MST treated/MST control × 100 was calculated. A T/C of 125 or over is considered to indicate significant tumor inhibition.

Results and Discussion

The activities of all of the compounds listed in this series have been compared in the in vivo P-388 and L-1210 leukemia systems in mice, and the results obtained are given in Table 1. This table also gives the tissue culture cytotoxicity obtained against HeLa cells in vitro. These data had been reported previously [3, 4].

It can be seen that only those compounds in this series in which the substitution at R_3 is acetoxy have appreciable activity in the in vivo P-388 test as determined by MED (minimum effective dose). However, when this substitution at R_3 is combined with an acetoxy at R_1, the potency is very low. The ranking of these compounds based on P-388 activity is 15-acetoxyscirpenol (V) > 4,15-diacetoxyscirpenol (IV) > 3,4,15-triacetoxyscirpenol (I) > 3,4-diacetoxyscirpenol (III) = 4-ace-

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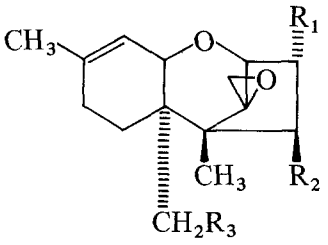

Fig. 1. Structures of anguidine and derivatives

Number	Name	R_1	R_2	R_3
I	3,4,15-Triacetoxyscirpenol	$OCOCH_3$	$OCOCH_3$	$OCOCH_3$
II	3,15-Diacetoxyscirpenol	$OCOCH_3$	OH	$OCOCH_3$
III	3,4-Diacetoxyscirpenol	$OCOCH_3$	$OCOCH_3$	OH
IV	4,15-Diacetoxyscirpenol (anguidine)	OH	$OCOCH_3$	$OCOCH_3$
V	15-Acetoxyscirpenol	OH	OH	$OCOCH_3$
VI	4-Acetoxyscirpenol	OH	$OCOCH_3$	OH
VII	3-Acetoxyscirpenol	$OCOCH_3$	OH	OH
VIII	Scirpenetriol	OH	OH	OH

Table 1. In vitro and in vivo activity of anguidine and derivatives

Compound[a]	HeLa activity ED_{50}[b]	P-388 Leukemia dose mg/kg			L-1210 Leukemia dose mg/kg		
		Max. T/C	OD[c]	MED[c]	Max. T/C	OD[c]	Med[c]
I	0.036	144	0.8	0.2	167	1.6	0.4
II	0.47	133	3.2	3.2	133	1.6	1.6
III	1.91	128	1.6	1.6	121	3.2	–
IV	0.002	211	3.2	0.1	167	1.6	0.2
V	0.005	233	0.8	0.0125	157	0.4	0.1
VI	0.074	133	6.4	1.6	113	>3.2	–
VII	>0.3	133	6.4	6.4	122	6.4	6.4
VIII	0.088	139	6.4	1.6	143	0.8	0.4

[a] From Figure 1

[b] Dose (μg/ml) required to cause a 50% reduction in net protein production

[c] OD = optimal dose; MED = minimum effective dose

toxyscirpenol (VI) = scirpenetriol (VIII) > 3,15-diacetoxyscirpenol (II) > 3-acetoxyscirpenol (VII).

Thus it appears, at least in the in vivo P-388 leukemia test in mice under the conditions in our laboratory, that the 15-acetoxy function (R_3) is required for good activity, but substitution at position 3 (R_1) is more deleterious to activity than substitution at position 4 (R_2). Therefore, if we rank the three monoacetoxyscirpenols, the 15-acetoxyscirpenol (V) > 4-acetoxyscirpenol (VI) > 3-acetoxyscirpenol (VII).

The same rank order for all of the compounds, although not as clearly defined, holds for the comparison in the L-1210 lymphatic leukemia test, the 15-acetoxyscirpenol again being the most effective. However, scirpenetriol in this test ranks closer to anguidine and to the triacetoxy analog.

The superior activity of 15-acetoxyscirpenol (V) in the in vivo P-388 leukemia test has been the subject of a separate communication [4]. The MED of this compound is approximately eightfold less than that of the parent anguidine (IV), some 16fold less than that of the trisubstituted 3,4,15-triacetoxyscirpenol (I), and over 200fold less than that of 3,15-diacetoxyscirpenol (II).

The necessity of an acyloxy substitution at position 15 for appreciable in vivo P-388 activity has been borne out through the preparation of other derivatives of the 12,13-epoxytrichothecenes. The preparation and activities of compounds in this series will be the subject of a separate communication (T. W. Doyle et al., to be published).

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