STRUCTURE-ACTIVITY RELATIONSHIPS FOR UNSATURATED DIALDEHYDES

3. MUTAGENIC, ANTIMICROBIAL, CYTOTOXIC, AND PHYTOTOXIC ACTIVITIES OF MERULIDIAL DERIVATIVES

HEIDRUN ANKE, OLOV STERNER† and WOLFGANG STEGLICH††

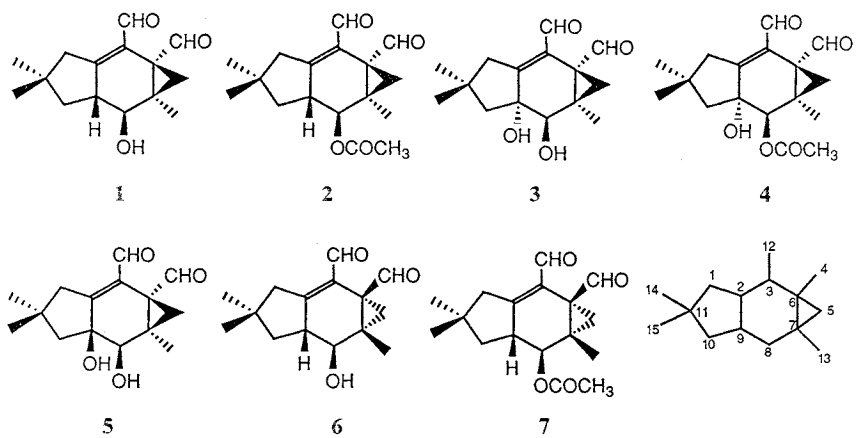
Departments of Biotechnology, University of Kaiserslautern,
D-6750 Kaiserslautern, FRG
†Organic Chemistry, Lund Institute of Technology,
P.O.B. 124, S-221 00 Lund, Sweden
††Organic Chemistry, University of Bonn,
Gerhard-Domagk-str. 1, D-5300 Bonn, FRG

(Received for publication December 5, 1988)

The mutagenic activity in the AMES' *Salmonella* assay, the antimicrobial activities against bacteria, fungi, and algae, the cytotoxic activities against Ehrlich ascitic tumor cells and L1210 cells, and the phytotoxic activities against *Lepidium sativum* and *Setaria italica*, of the unsaturated dialdehyde merulidial and six acetylated, hydroxylated, and cyclopropane ring isomerized derivatives of merulidial, are compared. Some possible structure-activity relationships are discussed.

Merulidial (**1**) is an isolactarane sesquiterpene, isolated from ethyl acetate extracts of *Merulius tremellosus*[1]. It is an antibiotic and cytotoxic agent[2], as well as a mutagen in the AMES' *Salmonella* assay (AMES' test)[2,3], and the presence of an unsaturated dialdehyde functionality is believed to be important for the biological activities of merulidial (**1**). Similar terpenes, also with an unsaturated dialdehyde functionality, have been isolated from a number of natural sources[4,5], and many of them are efficient insect antifeedants and antimicrobial agents. However, a specific biological activity may vary considerably, also in a series of very similar unsaturated dialdehydes[3,6], and it is obvious that small structural changes influence their biological activity. With the general aim to study structure-

Scheme 1.



activity relationships for this biologically important class of compounds, we have by acetylation, hydroxylation, and cyclopropane ring isomerization converted merulidial (**1**) into six derivatives (shown in Scheme 1), and compared some biological activities of the compounds. The assays chosen are the AMES' *Salmonella* assay[7], the plate diffusion and serial dilution assays against several microorganisms, the metabolic inhibition test (MIT)[8] with Ehrlich ascitic tumor (ECA) cells and L1210 cells, and a seed germination test with *Lepidium sativum* and *Setaria italica*[9].

Materials and Methods

Spectroscopy

NMR spectra were recorded with a Varian XL-300 instrument, and the chemical shifts are given in ppm relative TMS. High resolution (HR)-MS (electron impact (EI), 70 eV) were recorded with an AEI MS 50 instrument.

Merulidial Derivatives

Merulidial (**1**) was isolated as described previously[1]. Acetylmerulidial (**2**) (mp 135~137°C) and 9-β-hydroxymerulidial (**5**) were prepared as described in the same paper[1]. 9-α-Hydroxymerulidial (**3**) and 9-α-hydroxyacetylmerulidial (**4**) were prepared as described in ref 10. Isomerulidial (**6**) was prepared by ethanolysis at 25°C of acetylisomerulidial (**7**) in 1 mM NaOEt - EtOH. The poor yield of this reaction, only 1~2%, has limited the amounts of isomerulidial (**6**) available in this investigation. It was obtained as a colorless oil after chromatography on SiO_2: $[\alpha]_D^{22}$ +20° (*c* 0.1, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 9.86 (1H, s, 12-H), 9.29 (1H, s, 4-H), 3.16 (1H, d, $J_{8,9}$=10.1 Hz, 8-H), 3.03 (1H, m, 9-H), 2.86 (1H, dd, $J_{1a,1b}$=18.1 Hz, $J_{1a,10a}$=2.1 Hz, 1-H_a), 2.60 (1H, dd, $J_{1a,1b}$=18.1 Hz, $J_{1b,9}$=2.4 Hz, 1-H_b), 2.08 (1H, d, $J_{5a,5b}$=4.7 Hz, 5-H_a), 1.92 (1H, ddd, $J_{1a,10a}$=2.1 Hz, $J_{9,10a}$=8.0 Hz, $J_{10a,10b}$=12.7 Hz, 10-H_a), 1.31 (1H, dd, $J_{9,10b}$=10 Hz, $J_{10a,10b}$=12.7 Hz, 10-H_b), 1.28, 1.17, and 1.08 (3×3H, 3s, 13-H_3, 14-H_3, and 15-H_3), 1.03 (1H, d, $J_{5a,5b}$=4.7 Hz, 5-H_b); MS *m/z* 248.1425 (M, $C_{15}H_{20}O_3$, calcd for $C_{15}H_{20}O_3$: 248.1412), 230 (M−H_2O), 219 (M−CHO). Acetylisomerulidial (**7**) was obtained as a colorless oil by isomerization of acetylmerulidial (**2**) at 170°C in toluene for 3 hours[11]: $[\alpha]_D^{22}$ −75° (*c* 1.5, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 9.86 (1H, s, 12-H), 9.29 (1H, s, 4-H), 4.14 (1H, d, $J_{8,9}$=11.0 Hz, 8-H), 3.17 (1H, m, 9-H), 2.86 (1H, dd, $J_{1a,1b}$=18.3 Hz, $J_{1a,10a}$=2.0 Hz, 1-H_a), 2.61 (1H, dd, $J_{1a,1b}$=18.3 Hz, $J_{1b,9}$=3.0 Hz, 1-H_b), 2.07 (1H, d, $J_{5a,5b}$=5.2 Hz, 5-H_a), 1.82 (1H, ddd, $J_{1a,10a}$=2.0 Hz, $J_{9,10a}$=8.0 Hz, $J_{10a,10b}$=12.7 Hz, 10-H_a), 1.19 (1H, dd, $J_{9,10b}$=10 Hz, $J_{10a,10b}$=12.7 Hz, 10-H_b), 1.18 (1H, d, $J_{5a,5b}$=5.2 Hz, 5-H_b), 1.15, 1.12, and 1.09 (3×3H, 3s, 13-H_3, 14-H_3, and 15-H_3); MS *m/z* 290.1514 (M, $C_{17}H_{22}O_4$, calcd for $C_{17}H_{22}O_4$: 290.1518), 230 (M−CH_3COOH).

Biological Assays

The AMES' *Salmonella* assay was performed according to the standard procedure[7], with the two frameshift mutant strains TA98 and TA2637 and the base-pair substitution mutant strain TA100 in the absence of metabolic activation (S9 mix). (The mutagenic activity of the compounds assayed here, as well as for unsaturated dialdehydes in general[3], is reduced by the presence of S9.) The solvent used throughout was acetone, and as a solvent control, blanks were run with acetone only. All plates were triplicated, and all assays were repeated at least once with very similar results. The average number of spontaneous revertants per plate was for TA98 between 35 and 41; for TA2637 between 47 and 61; and for TA100 between 124 and 153. 1-[(Diethylaminoethyl)amino]-4-(methanesulfonyloxymethyl)thioxanthen-9-one, kindly provided by Winthrop Ltd., was used to determine the sensitivity of the strains. 100 μg of this compound per plate gave 480 colonies for TA98, 710 for TA2637, and 650 for TA100 (inclusive of the spontaneous background). The primary data from each run were subjected to analysis by linear regression (assuming a linear dose-response relationship), and a positive correlation is considered to be at hand when the correlation coefficient $r>0.6$. When $r<0.6$ the mutagenic response in Table 1 is recorded as 0. For the antimicrobial assays, the bacteria were grown in nutrient broth, the fungi in yeast - malt - glucose medium[12], and the same media was used

for the evaluation of the antimicrobial activity. ECA cells and L1210 cells (ATCC) were grown as described earlier[13]. The seed germination assay was carried out as described in ref 9.

Results and Discussion

The results presented in Tables 1 to 5 show that the biological activities of the assayed compounds

Table 1. The mutagenic activity in the *Salmonella*/microsome assay of compounds **1**~**7** (strains TA98, TA2637, and TA100, in the absence of S9 mix).

Compound	μg/plate[a]	Mutagenic response[b]			Mutagenic activity[c]		
		TA98	TA2637	TA100	TA98	TA2637	TA100
1	50	126 (0.92)[d]	56 (0.95)	305 (0.97)	0.62	0.28	1.5
2	600	0	0	0	0	0	0
3	100	177 (0.97)	134 (0.97)	1,095 (1.00)	0.47	0.35	2.9
4	400	0	0	219 (0.97)	0	0	0.17
5	60	121 (0.98)	28 (0.81)	293 (0.99)	0.53	0.12	1.3
6	20[e]	0	0	0	0	0	0
7	200	0	0	0	0	0	0

[a] The highest non-toxic concentration (except for compound **6**, see below).
[b] The mutagenic response is recorded as the number of colonies in excess of the solvent control (background has been subtracted) at the given concentration.
[c] The mutagenic activity is given by the slope of the dose-response curve in number of excess revertants per nmol.
[d] The correlation coefficients are given in parenthesis.
[e] Maximal concentration with the amounts of compound **6** available.

Table 2. MICs of **1**~**5** and **7** against bacteria and yeasts in the serial dilution assay (10^6 cells/ml).

Organism	MIC (μg/ml)					
	1	**2**	**3**	**4**	**5**	**7**
Bacteria						
Acinetobacter calcoaceticus	50	50	100	100	>100	>50
Bacillus brevis	30	30	100	>100	>100	>50
B. subtilis	50	50	>100	>100	>100	>50
Micrococcus luteus	50	50	>100	>100	>100	>50
Yeasts						
Nematospora coryli	5	1	100	>100	100	50
Saccharomyces cerevisiae	50	20	>100	>100	>100	>50
S. cerevisiae iS1	10	5	>100	>100	>100	>50

Table 3. Antifungal and aligicidal activity of **1**~**5** and **7** in the plate diffusion assay.

Organism	Inhibition zone (mm)					
	1 10/50	**2** 10/50	**3** 50	**4** 50	**5** 50	**7** 50
Chlorella vulgaris	15/25	—/15	—	—	—	10
Mucor miehei	14/16	15/18	—	—	—	13
Paecilomyces variotii	—/8	—/10	—	—	—	—
Penicillium notatum	—/7	—/10	—	—	—	—

Filter discs (6 mm diameter) bearing 10 or 50 μg of the compounds were placed onto the agar seeded with the test organism.
—: Indicates that no inhibition zone was detected.

vary considerably, also between different assays. For example, the mutagenic activities in the AMES' *Salmonella* assay (Table 1) of merulidial (**1**) and 9-α-hydroxymerulidial (**3**) are diminished by acetylation of their C-8 hydroxyl groups (to form the acetylated derivatives **2** and **4**, respectively). (Merulidial (**1**) has previously been assayed for mutagenic activity[3], but was reassayed in this investigation in order to get comparable results. The somewhat different response observed here with strain TA98 is believed to be due to a change in the sensitivity to our specimen of TA98.) However, no similar differences are observed between compounds **1** and **3** and their acetylated derivatives **2** and **4** in the other assays (Tables 2 to 5), the antifungal activity of merulidial (**1**) is instead increased by acetylation, and this is what one would expect as the result of acetylation. These observations indicate that the molecular mechanism responsible for the mutagenicity of merulidial (**1**) is different from the mechanisms resulting in for instance antimicrobial and cytotoxic activity. It was recently shown[14] that the two unsaturated dialdehydes isovelleral (**8**) and polygodial (**9**) (Scheme 2) react with methylamine in aqueous solution, and the products formed were suggested to be the pyrrole derivatives **10** and **11** (formed after addition of water to the double bonds and the cyclopropane ring, see Scheme 2). Although both compounds are potent antimicrobial agents[5,15], isovelleral (**8**) is a potent mutagen in the AMES' *Salmonella* assay while polygodial (**9**) is not[3]. The major difference between the two compounds is the presence of a conjugated cyclopropane ring in isovelleral (**8**), which has to be opened if the mutagenicity of isovelleral (**8**) in AMES' test involves the formation of a pyrrole derivative corresponding to compound **10**. The electrophile formed during such a hypothetical opening could be essential for the mutagenic activity of isovelleral (**8**), as it, instead of reacting with water as depicted in compound **10**, could react with the genetic material and thereby lead to cross-links in the DNA. If the same reasoning is applied on the compounds assayed here, this could explain the difference in mutagenicity between, for example, merulidial (**1**) and acetylmerulidial (**2**). If the electrophilic intermediate shown in Fig. 1, with a positive charge developing on C-7, is formed (from acetylmerulidial (**2**) and an endogenous primary amine R–NH_2), it could be intercepted by its own acetyl group (as suggested in Fig. 1) whereby it would be excluded from reactions with endogenous nucleophiles. The shortest distance between the carbonyl oxygen in the

Table 4. Cytotoxic activity of **1**~**5** and **7** against ECA and L1210 cells.

Cell line	Compound	Concentration (μg/ml) needed for	
		50% reduction	Lysis
ECA	**1**	20	50
	2	10	20
	3	30	50
	4	>50	>50
	5	50	>50
	7	>50	>50
L1210	**1**	10	50
	2	10	20
	3	30	>50
	4	>50	>50
	5	50	>50
	7	30	>50

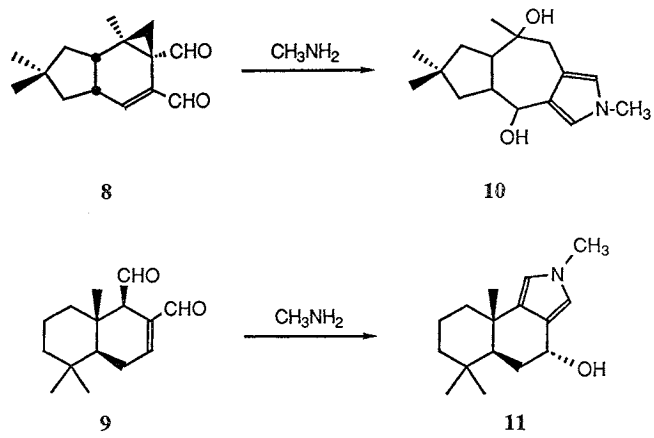
Concentrations needed for a 50%-reduction of cell number and lysis in comparison with a control (without inhibitors).

Table 5. Phytotoxic activity of **1**~**5** and **7** against *Lepidium sativum* and *Setaria italica*.

Plant	Inhibitory concentration (μg/ml)					
	1	**2**	**3**	**4**	**5**	**7**
L. sativum	50	50	>100	>100	>100	>100
S. italica	50	50	100	100	>100	>100

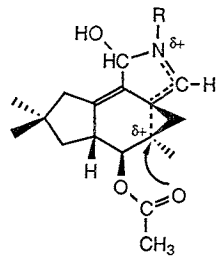
Concentrations needed for complete inhibition of seed germination.

Scheme 2.



acetyl group and C-7 in the most stable conformer of acetylmerulidial (**2**) is only 2.39 Å, compared to 1.42 Å in a normal C–O bond. (The distances were obtained from structures minimized as described previously[16], assuming free rotation of the acetyl group.) The sensitivity of the AMES' *Salmonella* strains to mutagens is demonstrated by the toxicity of the mutagenic compounds **1**, **3**, and **5** towards the strains. The highest non-toxic concentrations were found to be 2.5~5 μg/ml (corresponding to 50~100 μg/plate), which is considerably lower than the concentrations required to inhibit other bacteria (see Table 2).

Fig. 1. The interception by its own acetyl group, of the hypothetical intermediate formed when acetylmerulidial (**2**) reacts with a primary amine (see text for further details).



Although hydroxylation of merulidial (**1**) to 9-α-hydroxymerulidial (**3**) and 9-β-hydroxymerulidial (**5**), as well as of acetylmerulidial (**2**) to 9-α-hydroxyacetylmerulidial (**4**), does not affect the mutagenic activity very much, it reduces the antimicrobial, cytotoxic, and phytotoxic activity dramatically. The same phenomena have been observed earlier, hydroxylation of unsaturated dialdehydes may actually increase the mutagenic activity[3] while it always appears to decrease the antimicrobial activity[9,17]. These differences may be due to a less effective uptake of the more polar hydroxylated derivatives, the *Salmonella* strains used in AMES' test being an exception as they are designed not to restrict compounds from entering the cells[7].

Unfortunately, it was not possible to prepare sufficient amounts of isomerulidial (**6**), and only limited data, from AMES' test, were obtained. Due to the small amounts available, the highest concentration assayed was 20 μg/plate (corresponding to 1 μg/ml). At this concentration, no toxic or mutagenic effects could be observed. If isomerulidial (**6**) is as mutagenic as merulidial (**1**), one would clearly have seen a response at this concentration, and it is therefore possible to conclude that isomerulidial (**6**) is less active than merulidial (**1**) in AMES' test. When the two acetylated isomers **2** and **7** are compared, it is obvious that acetylmerulidial (**2**) is the more active (especially antifungal) compound. It is somewhat unexpected that isomerization of the cyclopropane ring affects the biological

activity, but the similar thing has been observed for isovelleral (**8**) and its isomer[3].

The impression that the biological activities of unsaturated dialdehydes are influenced also by very small structural changes, is supported by the results presented in this investigation. Further knowledge about structure-activity relationships for this biologically important functionality will hopefully be obtained by the computational correlation of these and other data, with structural parameters of unsaturated dialdehydes[16].

Acknowledgments

We thank A. HELFER, Kaiserslautern, for expert technical assistance, Dr. F. BOCCHIO, Lund, for performing the molecular mechanics calculations, and Dr. G. ECKHARDT, Bonn, for recording the high resolution mass spectra. Support from the Alexander von Humboldt foundation (FRG) and the Swedish Natural Science Research Council for O.S. is gratefully acknowledged. All *Salmonella* strains were kindly provided by Prof. BRUCE AMES, University of California, Berkeley, U.S.A.

References

1) GIANNETTI, B.; B. STEFFAN, W. STEGLICH, W. QUACK & T. ANKE: Antibiotics from basidiomycetes. 23. Merulidial, an isolactarane derivative from *Merulius tremellosus*. Tetrahedron 42: 3579~3586, 1986
2) QUACK, W.; T. ANKE, F. OBERWINKLER, B. M. GIANNETTI & W. STEGLICH: Antibiotics from Basidiomycetes. V. Merulidial, a new antibiotics from the Basidiomycete *Merulius tremellosus* FR. J. Antibiotics 31: 737~741, 1978
3) STERNER, O.; R. CARTER & L. NILSSON: Structure-activity relationships for unsaturated dialdehydes. 1. The mutagenic activity of 18 compounds in the Salmonella/microsome assay. Mutat. Res. 188: 169~174, 1987
4) CIMINO, G.; S. DE ROSA, S. DE STEFANO, G. SODANO & G. VILLANI: Dorid nudibranch elaborates its own chemical defense. Science 219: 1237~1238, 1983
5) STERNER, O.; R. BERGMAN, J. KIHLBERG & B. WICKBERG: The sesquiterpenes of *Lactarius vellereus* and their role in a proposed chemical defense system. J. Nat. Prod. 48: 279~288, 1985
6) CAPRIOLI, V.; G. CIMINO, R. COLLE, M. GAVAGNIN, G. SODANO & A. SPINELLA: Insect antifeedant activity and hot taste for humans of selected natural and synthetic 1,4-dialdehydes. J. Nat. Prod. 50: 146~151, 1987
7) AMES, B.; J. MCCANN & E. YAMASAKI: Methods for detecting carcinogens and mutagens with the Salmonella/microsome mutagenicity assay. Mutat. Res. 31: 347~365, 1975
8) EKWALL, B.: Screening of toxic compounds in mammalian cell cultures. Ann. N.Y. Acad. Sci. 407: 64~77, 1983
9) ANKE, H.; E. HILLEN-MASKE & W. STEGLICH: 9-β-Hydroxymarasmic acid and other sesquiterpenoids from submerged cultures of a basidiomycete. Z. Naturforsch. C 44: 1~6, 1989
10) STERNER, O. & W. STEGLICH: Oxidation of the fungal sesquiterpene merulidial on alumina gel. Liebigs Ann. Chem. 1988: 823~824, 1988
11) WICKBERG, B.; R. BERGMAN, T. HANSSON & O. STERNER: A total synthesis of the mushroom sesquiterpene (+)-isovelleral via a diastereoselective intramolecular Diels-Alder reaction and an unusual pericyclic rearrangement. Paper of the 1st Princess Chulabhorn Congress on Natural Products, Bangkok, 1987
12) ANKE, H.; J. CASSER, W. STEGLICH & E.-H. POMMER: Antibiotics from basidiomycetes. 26. Phlebiakauranol aldehyde an antifungal and cytotoxic metabolite from *Punctularia atropurpurascens*. J. Antibiotics 40: 443~449, 1987
13) LEONHARDT, K.; T. ANKE, E. HILLEN-MASKE & W. STEGLICH: 6-Methylpurine, 6-methyl-9β-D-ribofuranosylpurine, and 6-hydroxymethyl-9β-D-ribofuranosylpurine as antiviral metabolites of *Collybia maculata*. Z. Naturforsch. C 42: 420~424, 1987
14) CIMINO, G.; G. SODANO & A. SPINELLA: Correlation of the reactivity of 1,4-dialdehydes with methylamine in biomimetic conditions to their hot taste: Covalent binding to primary amines as a molecular mechanism in hot taste receptors. Tetrahedron 43: 5401~5410, 1987
15) TANIGUCHI, M.; T. ADACHI, S. OI, A. KIMURA, S. KATSUMURA, S. ISOE & I. KUBO: Structure-activity relationship of the *Warburgia* sesquiterpene dialdehydes. Agric. Biol. Chem. 48: 73~78, 1984
16) NILSSON, L.; R. CARTER, O. STERNER & T. LILJEFORS: Structure-activity relationships for unsaturated

dialdehydes. 2. A PLS correlation of theoretical descriptors for six compounds with mutagenic activity in the Ames Salmonella assay. Quant. Struct.-Act. Relatsh. 7: 84~91, 1988

17) HEIM, J.; T. ANKE, U. MOCEK, B. STEFFAN & W. STEGLICH: Antibiotics from basidiomycetes. XXIX. Pilatin, a new antibiotically active marasmane derivative from cultures of *Flagelloscypha pilatii* Agerer. J. Antibiotics 41: 1752~1757, 1988