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cis-DIHYDRODIOLS MICROBIALLY PRODUCED FROM HALO- AND METHYLBENZOIC ACIDS

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Abstract—The preparation of thirteen halo- and methyl-substituted 3,5-cyclohexadiene-1, 2-diol-1-carboxylic acids from microbial oxidation of the corresponding benzoic acids is reported. The properties, including CD-, UV-, MS-spectra are compared. The *cis*-configuration of the glycol group is determined by acetonide formation with 2,2-dimethoxypropane and from spectroscopic data.

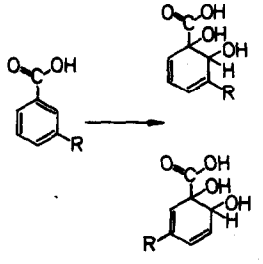
Vicinal-dihydrodiols have been known as early intermediates in the metabolism of nonphenolic aromatic hydrocarbons since 1935.[1] Bacterial catabolism is initiated by double hydroxylation yielding *cis*-configurated dihydrodiols,[2] whereas mammalian oxidation of aromatic compounds proceeds via monooxygenase catalysed reaction to arene oxides which are subsequently hydrated to *trans*-dihydrodiols.[3] From bacteria vic-*cis*-dihydrodiols have been identified as the initial oxidation products of benzene,[4] toluene,[5] ethylbenzene,[6] biphenyl,[7] 5 - amino - 4 - chloro - 2 - phenyl - 3(2H)pyridazinone,[8] naphthalene,[9] benzo[a]pyrene,[10] xylene,[11] dibenzothiophene,[12] p-cymene,[13] and anthracene.[14] Reiner and Hegeman[15] have recently demonstrated that a previously unknown compound, 3, 5 - cyclohexadiene - 1, 2 - diol - 1 - carboxylic acid (DHB[c]), is an intermediate in the conversion of benzoic acid to catechol. Evidence presented by Reiner[16] shows that this compound is a general intermediate in the metabolism of benzoic acid via catechol by bacteria.

We are currently analyzing the reasons for the biological persistence of halo-substituted aromatic compounds using halogenated benzoic acids as model compounds. In the present paper we want to characterize several halogen- and methyl-substituted 3, 5 - cyclohexadiene - 1, 2 - diol - 1 - carboxylic acids as the initial products of oxygenation of substituted benzoic acids. Moreover these compounds have been studied as substrates of the DHB dehydrogenase which is the subsequent enzyme in benzoic acid catabolism.[17]

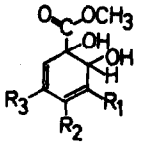
RESULTS AND DISCUSSION

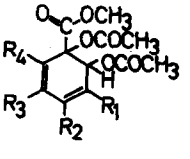
The mutant strain *Alcaligenes eutrophus* B 9 from Reiner and Hegeman[15] was found to be defective in the DHB dehydrogenase. Induced cells cooxidized benzoic acid to DHB in quantitative yield. The ability to convert substituted benzoic acids into the corresponding 3, 5 - cyclohexadiene - 1, 2 - diol - 1 - carboxylic acids depends on the specificity of the benzoate 1, 2 - dioxygenase.[17] Relative high turnover rates were found with *meta*-substituted benzoic acids. *Ortho*- and *para*-substituted benzoic acids were cooxidized with considerable rates only with fluorine as a substituent. As mentioned by Dorn *et al.*[18] two isomeric chloro-DHBs were formed when 3-chlorobenzoic acid was degraded by *Pseudomonas sp.* B 13.

Reiner and Hegeman[15] detected 3-methyl-, 5-chloro- and 3-fluoro-DHB as the only cooxidation products from the corresponding *meta*-substituted benzoic acids. In contrast we found according to Scheme 1 both 3- and 5-substituted DHBs from all *meta*-substituted benzoic acids. The isomers could be separated directly by reverse phase HPLC or by TLC as methylesters. We were able to isolate 3-chloro-, 5-chloro-, 3-bromo-, 5-bromo-, 3-methyl-, 3-fluoro-, 4-fluoro-, 5-fluoro- and 6-fluoro-DHB in good yield. 4-Chloro-, 3,5-dichloro-, 4-methyl- and 5-methyl-DHB were accessible only with difficulty.



Scheme 1. 3- and 5-substituted 3, 5 - cyclohexadiene - 1, 2 - diol - 1 - carboxylic acids from 3-substituted benzoates (R = halogen or Me).





1a; $R_1 = R_2 = R_3 = H$
2: $R_1 = Cl, R_2 = R_3 = H$
3: $R_1 = R_3 = H, R_2 = Cl$
4: $R_1 = R_2 = H, R_3 = Cl$
5: $R_1 = R_3 = Cl, R_2 = H$
6: $R_1 = Br, R_2 = R_3 = H$
7: $R_1 = R_2 = H, R_3 = Br$
8: $R_1 = CH_3, R_2 = R_3 = H$
9: $R_1 = R_3 = H, R_2 = CH_3$
10: $R_1 = R_2 = H, R_3 = CH_3$
1b: $R_1 = R_2 = R_3 = R_4 = H$
11: $R_1 = F, R_2 = R_3 = R_4 = H$
12: $R_1 = R_3 = R_4 = H, R_2 = F$
13: $R_1 = R_2 = R_4 = H, R_3 = F$
14: $R_1 = R_2 = R_3 = H, R_4 = F$

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[c]DHB = (−) - 3, 5 - cyclohexadiene - 1, 2 - diol - 1 - carboxylic acid (derived from the trivial name, dihydrodihydroxybenzoic acid).

Table 1. NMR data ($CDCl_3$) of 3,5 - cyclohexadiene - 1,2 - diol - 1 - carboxylic acid methylesters and some diacetyl derivatives

Compd.	CH(vinylic)	COH-H(allylic)	OH	$O-CH_3$	$C-CH_3$
1a	5.98-6.20 (1H, m) 5.66-5.95 (3H, m)	4.80 (1H, db)	3.92 (1H, s) 3.48 (1H, db)	3.82 (3H, s)	
2	5.94-6.18 (2H, m) 5.60-5.80 (1H, m)	4.74 (1H, s)	3.12 (1H, sb)	3.88 (3H, s)	
4	5.80-5.92 (3H, m)	4.84 (1H, s)	2.93 (1H, sb)	3.88 (3H, s)	
5	6.11 (1H, t) 5.80 (1H, m)	4.77 (1H, m)		3.90 (3H, s)	
6	6.38 (1H, dq) 5.70-6.10 (2H, m)	4.79 (1H, sb)	3.06 (1H, sb) 3.78 (1H, db)	3.90 (3H, s)	
7	5.70-6.14 (3H, m)	4.85 (1H, sb)	2.99 (1H, sb)	3.89 (3H, s)	
8	5.94-6.15 (1H, q) 5.53-5.81 (2H, m)	4.67 (1H, sb)	3.68 (1H, sb) 2.82 (1H, sb)	3.86 (3H, s)	1.90 (3H, s)
10	5.79 (2H, s) 5.47 (1H, m)	4.79 (1H, sb)	3.00 (1H, sb) 3.65 (1H, sb)	3.84 (3H, s)	2.02 (3H, s)

	CH(vinylic + allylic)	$OCOCH_3$	$O-CH_3$
1b	5.90-6.33 (4H, m) 4.68-5.90 (1H, m)	2.07 (3H, s) 2.10 (3H, s)	3.73 (3H, s)
11	5.53-6.38 (4H, m)	2.14 (3H, s) 2.16 (3H, s)	3.78 (3H, s)
12	6.02-6.18 (3H, m) 5.41 (1H, m)	2.06 (3H, s) 2.10 (3H, s)	3.74 (3H, s)
13	6.08 (1H, s) 5.70-6.02 (3H, m)	2.05 (3H, s) 2.08 (3H, s)	3.71 (3H, s)
14	5.64-6.20 (4H, m)	2.09 (3H, s) 2.16 (3H, s)	3.81 (3H, s)

Since the free acids of the DHBs decompose readily, the methylesters (**1a**, **2-10**) or their 1,2 - diacetyl derivatives (**1b**, **11-14**) were prepared for identification. The NMR spectrum of 3,5 - cyclohexadiene - 1,2 - diol - 1 - carboxylic acid showed very complex signals[15] with a multiplet between δ 5,6 and 6,2 ppm corresponding to the vinylic protons. Only the allylic proton gave a separated doublet at lower field. For all isolated DHB-methylesters the same complexity of the signals was found (Table 1). The bands of the methylprotons of the estergroup and of the allylic proton could be identified for all substituted DHB-methylesters. In compound **8** and **10** a signal in the region δ 1,9-2,02 ppm could be attributed to the protons of the methylsubstituent. The protons of the OH groups gave broad bands in the region δ 2, 8-3, 7 ppm disappearing on D_2O exchange. The bands of the allylic proton of the 1,2-diacetyl derivatives were masked by the complex multiplet of the vinylic protons. In compounds **11-14** couplings between protons and fluorine nuclei resulted in increased complexity of the multiplets of the vinylic protons. A clear assignment of the structures of the isomeric 3- and 5-substituted DHBs could not be based on the NMR data. Therefore the 3- and 5-substituted DHB-methylesters were rearomatisized to the corresponding salicylic acid methylesters and identified with authentic preparations (TLC, UV and IR spectra).

The UV data of the DHBs (Table 2) correspond to the assignment of the 3- and 5-substituted isomers. Typical substituent effects[19] on the $\pi \rightarrow \pi^*$ transition of the conjugated diene system were observed depending on the nature *and* position of the substituent on the conjugated double bonds. The strongest bathochromic shift was found with substituents at the end of the conjugation

Cross-conjugation of the diene system with the substituents in 4- or 5-position as being found in compound **3**, **4**, **7**, **9** and **10** generate considerable smaller bathochromic shifts. The spectrum of **5** shows that the effects of two substituents are additive.

A pronounced hyperchromic effect was found when a substituent is located at the end of the conjugated system (see compound **2**, **6** and **8**), in contrast to a hypochromic effect with substituents standing cross-conjugated (**4** and **7**). The low hyperchromic effect observed with **5** appears to be the sum of the substituent effects of the 3- and 5-substituted chromophore being found in **2** and **4**.

It has recently been demonstrated that in presence of an allylic oxygen in a skew diene the sign of the Cotton effect associated with the diene $\pi \rightarrow \pi^*$ transition is mainly determined by the helicity of the system C=C–C–O.[20] The CD data of vic-dihydrodiols are in agreement with these findings (Table 3). In vic-dihydrodiols with *trans*-configuration the helices by each of the allylic oxygens with its adjacent double bond have the same handedness. Consequently Cotton effects with high intensity must be observed. In contrast, the sense of the oxygen-containing helices in *cis*-cyclohexadiene-diols are opposed and counteract to each other. The resulting CD band in the 280 nm-region is rather weak and predominantly controlled by the skew sense of the nonplanar conjugated diene chromophore. In accordance

Table 2. Physical properties of 3,5-cyclohexadiene-1,2-diol-1-carboxylic acid methylesters and some diacetyl derivatives: [a]recrystallized from light petroleum (b.p. 40–60°); [b]recrystallized from methanol–water; [c]chloroform:acetone (80:20); [d]diisopropylether; [e]dichloromethane-light petroleum (80:20); [f]Nujol mull; [g]neat

Compd.	Molecular formula	Molecular ion (M^+) calcd.	found	m. p. (°C)	TLC (R_f x 100)		UV absorption λ_{max}^{MeOH}(nm)	ε	π→π* Cotton effect λ_{max}^{MeOH}(nm)	θ	IR absorption (cm^{-1}) ν_{OH}	ν_{CO}	$\nu_{C=C(conj.)}$
1a	$C_8H_{10}O_4$	170.0579	170.0589	51.5- 52.5[a]	21[c]	8[d]	260.5	3,100	284.5	-1,800	3,420	1,726	[f]
2	$C_8H_9O_4Cl$	204.0184	204.0187	86 - 87.5[a]	29[c]	18[d]	273	5,600	282	-16,900	3,355 3,425	1,716	1,577[f]
3	$C_8H_9O_4Cl$	204.0184	204.0189		28[c]	15[d]	265				3,440	1,750	[g]
4	$C_8H_9O_4Cl$	204.0184	204.0187	96.5- 97[a]	25[c]	13[d]	264	2,900	285	-1,500	3,340 3,440	1,718	1,630[f]
5	$C_8H_8O_4Cl_2$	237.9790	237.9797	113 -114[a]	34[c]	28[d]	276	4,400	286.5	-11,800	3,350 3,435	1,723	1,586[f] 1,630
6	$C_8H_9O_4Br$	247.9684	247.9693	102.5-103.5[a]	31[c]	18[d]	277	6,400	285	-17,400	3,380 3,438	1,718	1,574[f]
7	$C_8H_9O_4Br$	247.9684	247.9693	102.5-103.5[a]	26[c]	13[d]	266	2,600	285	-2,100	3,340 3,440	1,716	1,622[f]
8	$C_9H_{12}O_4$	184.0736	184.0738	90 - 91[a]	28[c]	12[d]	268	5,200	285.5	-5,600	3,360 3,450	1,718	1,590[f]
9	$C_9H_{12}O_4$	184.0736	184.0738		26[c]	9[d]	264				3,410	1,745	[g]
10	$C_9H_{12}O_4$	184.0736	184.0738		25[c]	8[d]	260.5				3,450	1,747	[g]
1b	$C_{12}H_{14}O_6$	254.0790	254.0786	91 - 91.5[b]	29[d]	15[e]	260.5	3,900	283	-8,500		1,745 1,768	1,645[f]
11	$C_{12}H_{13}O_6F$	272.0696	272.0708	84.5- 85[b]	32[d]	23[e]	267.5	3,550	270	-63,000		1,770	1,610[f] 1,696
12	$C_{12}H_{13}O_6F$	272.0696	272.0695	96 - 96.5[b]	33[d]	22[e]	264	3,550	269	-45,500		1,765	1,610[f] 1,679
13	$C_{12}H_{13}O_6F$	272.0696	272.0695	120 -121[b]	29[d]	13[e]	258	3,770	295	-1,000		1,761	1,614[f] 1,672
14	$C_{12}H_{13}O_6F$	272.0696	272.0711	65 - 66.5[b]	31[d]	21[e]	266	3,660	273	-49,000		1,760	1,612[f] 1,683

Table 3. $\pi \rightarrow \pi^*$ Cotton effects of vic-dihydro-dihydroxy-aromatic compounds: [a]ethanol; [b]solvent not given; [c]water; [d]methanol; [e]optical purity 25%; [f]optical purity not given.

Compound	Reference	Handedness of helices $C=C-C^1-O$	Handedness of helices $C=C-C^2-O$	Cotton effect predicted according to Beecham et al.[20a]	Long-wave length extremum λ(nm)	Long-wave length extremum θ
(-)-*trans*-1(R),2(R)-dihydroxy-cyclohexa-3,5-diene	35	left	left	strong, negative	258	-24,000[a,e]
(+)-*cis*-1(S),2(R)-dihydroxy-3-methylcyclohexa-3,5-diene	5c	right	left	weak	270	+ 2,000[b]
cis-1,2-dihydroxy-3-chlorocyclohexa-3,5-diene	36	oppose to each other		weak	280	+ 3,600[b]
(-)-*trans*-1(R),2(R)-dihydroxy-4-carboxycyclohexa-3,5-diene	20a	left	left	strong, negative	265	-15,000[c]
(+)-*trans*-1(S),2(S)-dihydroxy-3-carboxycyclohexa-3,5-diene	20a	right	right	strong, positive	273	+66,000[c]
(+)-*cis*-1(R),2(S)-dihydroxy-1,2-dihydronaphthalene	9b	left	right	weak	265	+ 9,580[d]
(-)-*trans*-1(R),2(R)-dihydroxy-1,2-dihydronaphthalene	35	left	left	strong, negative	262	-25,000[d,f]

with the UV data the CD spectra of the substituted DHB-methylesters show that the intensity of the diene-region Cotton effects depends on the nature *and* position of the substituent on the conjugated double bonds. When compound **1a** is compared with **4** and **7** only a small intensity change of the $\pi \rightarrow \pi^*$ transition results if C-5 bears a substituent. In contrast, a substituent at the end of the conjugated system strongly increases the negative Cotton effect (**2**, **6** and **8**). As shown by the more intense Cotton effects in compound **2** and **6** the diene is more strongly influenced by halogen substituents compared with the effect of the Me group in compound **8**.

The CD spectra of **2**, **4** and **5** (Fig. 1) indicate that the halogen substituents merely influence the chromophore without inducing conformational changes. The spectrum of **5** apparently reflects the additive electronic influences of the two chlorine substituents on the chromophore. Consequently the considerable differences between the spectrum of **2** and **4** cannot be based on the stabilization of different conformers, because only one of two conformations can be favoured when chlorine is present in 3- as well as in 5-position.

Since the cyclohexadien ring is almost planar a $\pi \rightarrow \pi^*$ Cotton effect of relative low intensity is observed. Most notably this band increases considerably by acetylation of the diol group as being found for **1b**. Higher steric hindrance of the acetoxy groups will increase the dihedral angle between the C-O-bonds. This will inevitably lead to higher optical activity, since the skew angle of the double bonds of the diene must increase, too. From the CD data (see Fig. 1 and Table 2) it can be deduced that all the DHBs obtained from cooxidation of substituted benzoic acids have the same configuration of the glycol group.

Most of the direct analytical methods for the determination of the relative and absolute stereochemistry of the vic-glycol group, i.e. ozonolysis of the cyclohexadiene ring to tartaric acids,[5c,21] NMR coupling analysis of vicinal protons,[22] chromogenic reaction with triacetylosmate,[23] and kinetic analysis of periodate fisson,[9b,24] cannot be applied to DHBs because one of the vicinal OH-bearing C atoms is substituted by a carboxylic group. Furthermore in each case only one, the *cis*-isomer was available.

For many cyclic vic-dihydroxy compounds two different ν-OH-bands have been detected in the 3μ-region, when diluted solutions in carbon tetrachloride were measured.[25] One of these bands corresponds to the O-H-stretching vibration due to an unbonded OH group whereas the other has been assigned to an intramolecularly H-bonded OH group. From the difference in the absorption frequency of the unbonded and bonded OH groups the strength of the H- bond has been estimated. The strongest H-bonding results when the OH groups are eclipsed or nearly eclipsed with a dihedral angle between the C-OH bonds being about O°. For the DHB-methylesters (**1a**, **2**, **4-8**) the difference in absorption frequency of the two OH-bands is 60 cm^{-1}, which corresponds to the value of $\Delta\nu = 61\ cm^{-1}$ determined by Kuhn for *cis*-cyclopentane-1, 2-diol where the dihedral angle is almost O°.[25a] From examinations of Dreiding models great differences of dihedral angles between the *cis*- and *trans*-isomers of DHBs must be assumed. The *cis*-isomer forms a minimum dihedral angle of O° when changing from one conformer into the other. On the other hand when the *trans*-glycol interconverts from the gauche to the anti-form the dihedral angle can never be smaller than 60°. From the high $\Delta\nu$-values it can be deduced that the OH groups in the DHBs are in a *cis*-configuration.

In addition the *cis*-configuration of the glycol group is supported by the reaction with 2,2-dimethoxypropane yielding an isopropylidene derivative. Böeseken had observed that exclusively the *cis*-isomer of cyclohexane-1, 2-diol formed an isopropylidene derivative.[26] The initial oxidation product of naphthalene by bacteria is a *cis*-1, 2-dihydrodiol which readily formed an acetonide with 2,2-dimethoxypropane.[9b] In contrast only naphthols were produced when the *trans*-dihydrodiol from naphthalene was treated with 2,2-dimethoxypropane under acidic conditions.[9b] The *cis*-configuration of the diol group in DHB is consistent with the fact that both atoms of molecular oxygen are incorporated into DHB.[15]

The exact masses of the molecular peak and the

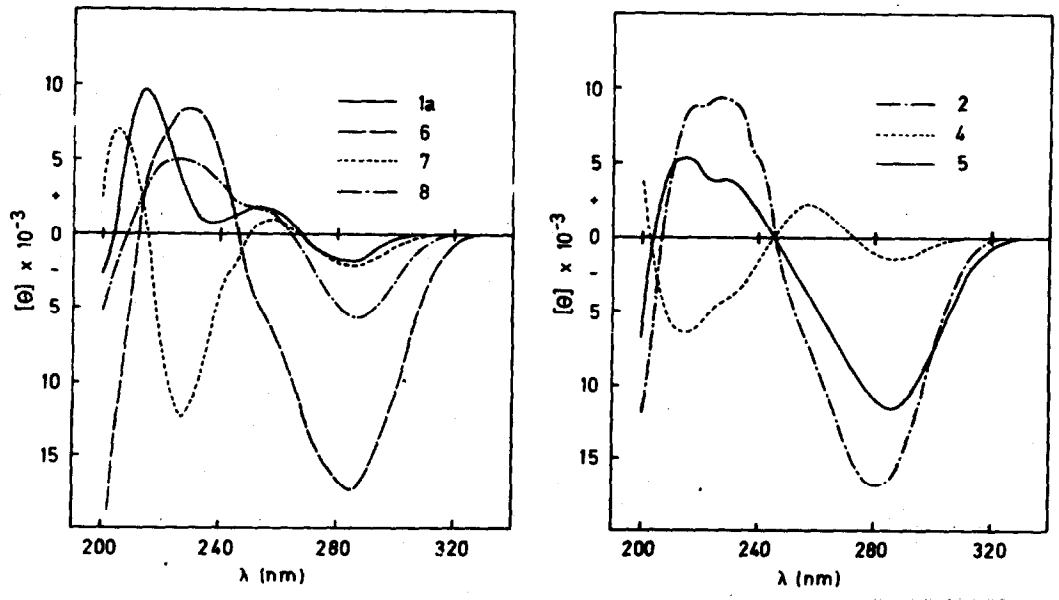
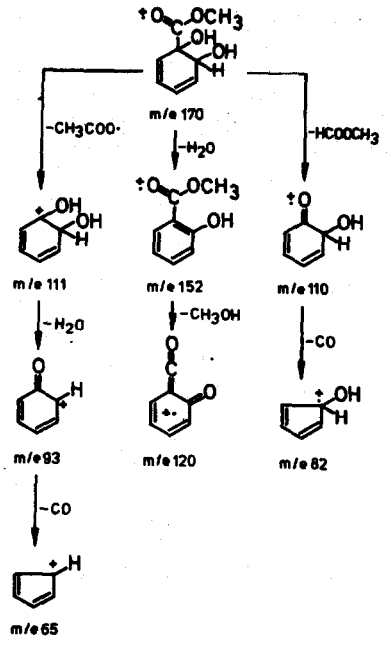
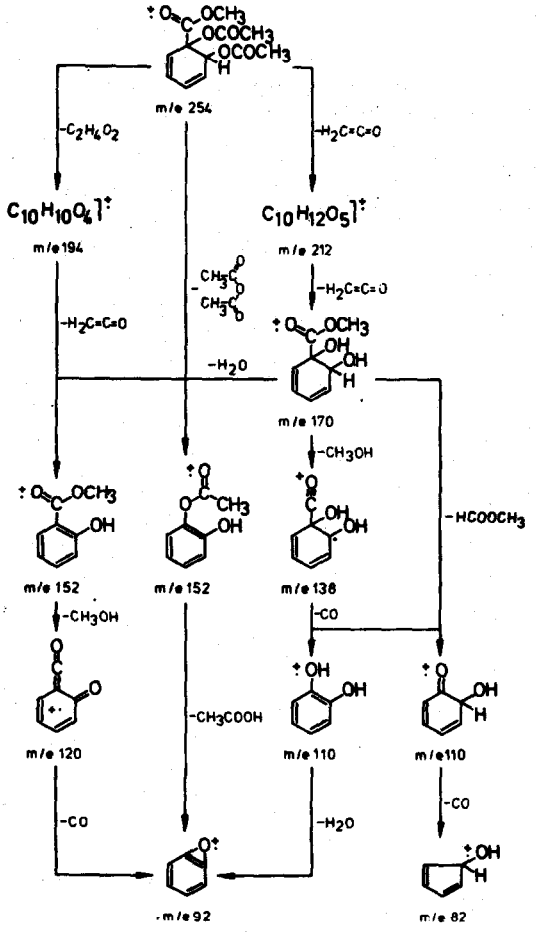


Fig. 1. CD spectra (in methanol) of (−)-3,5-cyclohexadiene-1,2-diol-1-carboxylic acid methylester (**1a**) and its 3-chloro-, 5-chloro, 3,5-dichloro-, 3-bromo-, 5-bromo- and 3-methyl-substituted analogues (**2**, **4**, **5**, **6**, **7** and **8**).



Scheme 2. MS fragmentation of 3,5-cyclohexadiene-1,2-diol-1-carboxylic acid methylester (1a).



Scheme 3. MS fragmentation of 1,2-diacetoxy-3,5-cyclohexadiene-1-carboxylic acid methylester (1b).

strongest fragments of compound **1a** and **1b** were determined by high resolution measurements, to give the chemical formula of the peaks. The parent peaks of the fragment peaks were determined by variable accelerator measurements. The main fragmentation pathways of compound **1a** are shown in Scheme 2. The loss of water from the molecular ion, which is a common feature of the mass spectra of vic-dihydrodiols,[4b, 5a, 9a, 10, 27, 28] leads to the peak at *m/e* 152. This fragment decomposes into *m/e* 120. For aromatic esters having a group with hydrogen in the ortho-position such a rearrangement fragment like that at *m/e* 120 is characteristic.[29, 30] The main fragmentation of the 1,2-diacetyl derivative **1b** is the elimination of Ac_2O from the molecular ion *m/e* 254 resulting in the basepeak *m/e* 152. Other peaks arise preponderantly from elimination of neutral molecules like CO, H_2O, CH_3OH, $CH_2{=}C{=}O$, CH_3COOH or $HCOOCH_3$. The fragmentations are summarized in Scheme 3. The spectra of all substituted DHBs (Fig. 2) suggest that the fragmentation pathways are analogous.

EXPERIMENTAL

Analytical methods. UV spectra were measured in MeOH on a Zeiss DMR 21 recording spectrophotometer. CD curves were determined at 20° with a Cary-Recording-Spectropolarimeter model 60 with Circular Dichroism Accessory 6003, using a cell with 20 mm pathlength. The spectra were measured in 1-5 mM solutions. IR spectra were recorded on a Pye-Unicam SP 1000 Spectrophotometer. Crystalline samples were mulled in Nujol and placed between NaCl disks. Non-crystalline and low melting samples were run on neat liquid films between NaCl disks. Low and high resolution mass spectra as well as the measuring of the metastable ions to determine the fragmentation pathways were made on a Du Pont mass spectrometer 21-492 at 70 eV and a source temp. of 100-200°. The NMR spectra were taken on a Varian HA 100 or Varian A-60 instrument with TMS as internal reference and the values are expressed in δ (ppm). M.ps (capillary method) are uncorrected. TLC analysis was performed on 0.25 mm layers of Kieselgel GF_{254} DC-Fertigplatten (E. Merck) and preparative TLC by using self prepared $20 \times 20 \times 0.1\ cm^3$ layers with Kieselgel 60 PF_{254} (E. Merck) on glass plates. The compounds were detected by observation under light of 254 nm wave length. Metabolites in the culture fluid were determined by high performance liquid chromatography using a 0.25 m column (Varian, Micro Pak C-H-column, particle size 10 μ, octadecylsilane, chemically bonded to LiChrosorb) and the solvent system 10 mM H_3PO_4 plus various concentrations of propanol-(2). A liquid chromatograph from Varian (Aerograph model 4200) and a Varian 254 nm UV spectrophotometer was used as a detector.

Microbial preparation of DHBs. Alcaligenes eutrophus mutant strain B 9 was provided by G. D. Hegeman. It was grown in 10 l-fermenter (B. Braun) in a succinate medium which has been described previously.[31] Cells were induced by the addition of 2 mM sodium benzoate at the end of the exponentiell growth phase. The culture was harvested by centrifugation after 4 hrs of induction, washed twice and suspended in 50 mM KH_2PO_4–Na_2HPO_4-buffer, pH 6.8. The induction could be controlled by HPLC by measuring the formation of DHB.

Conversion of benzoate, 2-fluoro-, 3-fluoro-, 4-fluoro-, 3-chloro-, 3-bromo- and 3-methylbenzoate to the corresponding DHBs was carried out in 3 l-fermenter (B. Braun) using a cell suspension corresponding to 0.15 mg protein/ml. The concentration of the substrate was maintained between 1 and 2 mM and controlled by HPLC at hourly intervals. Additional aliquots of benzoates were added if necessary. Because of substrate specificity of the benzoate 1,2-dioxygenase 4-chloro-, 3,5-dichloro- and 4-methylbenzoate were cooxidized very slowly. Only 0.2–1.0 mmoles of these substrates could be converted to DHBs, when the cell density given above was used. These substrates were incubated in fluted Erlenmeyer flasks on a rotary shaker at 30° for 24 hr.

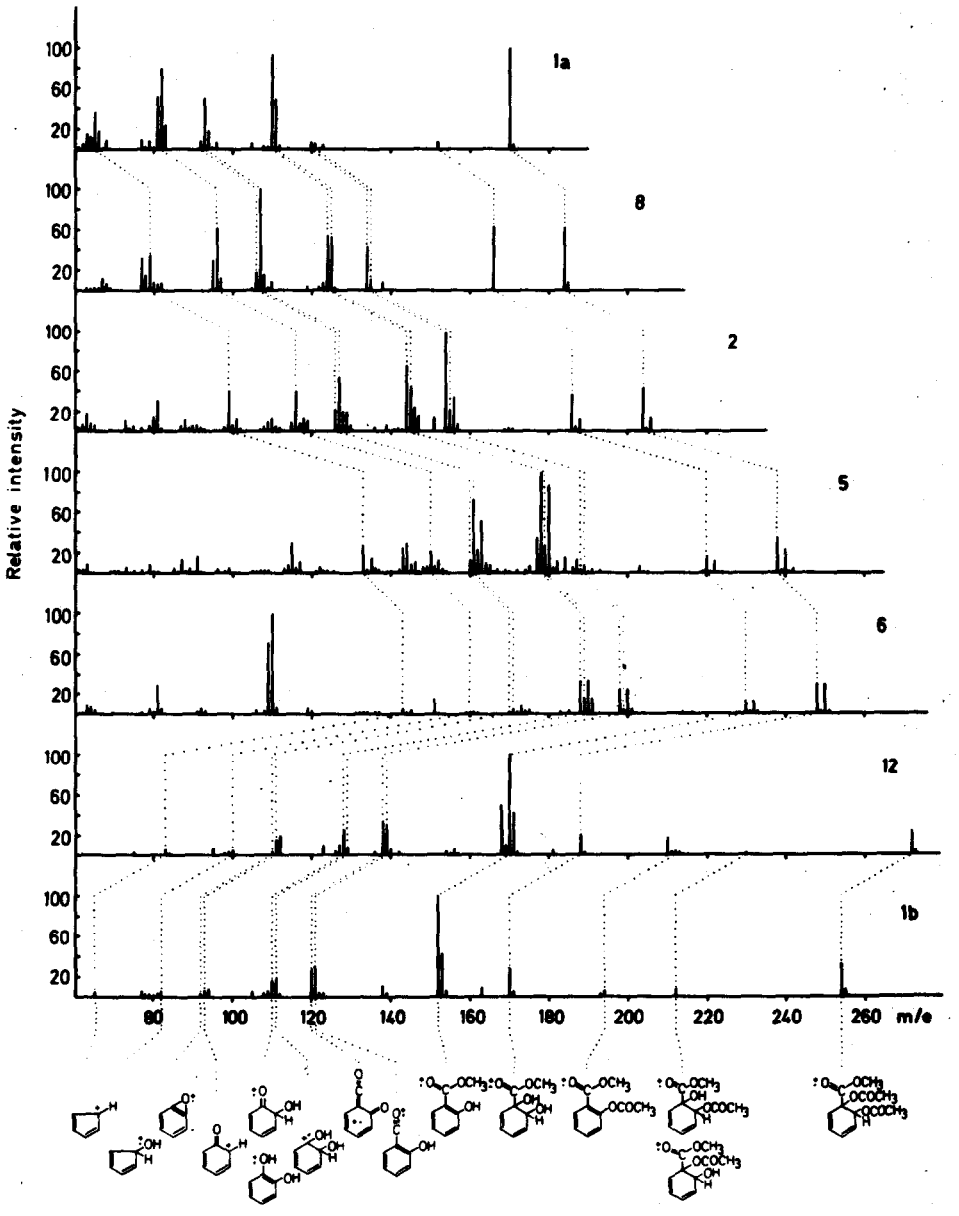


Fig. 2. MS-spectra of 3,5 cyclohexadiene-1,2-diol-1-carboxylic acid methylester (**1a**) its diacetylderivative (**1b**) and some typical substituted analogues (**2**, **5**, **6**, **8** and **12**).

The cell-free supernatant solutions containing the DHBs were concentrated 50-fold at 35–40°. The remaining solutions were extracted repeatedly with EtOAc at 0°, immediately after being acidified to pH 2 with HCl. The pooled EtOAc extracts were dried over $MgSO_4$ and concentrated to 10–20 ml by flash evaporation at 25–30°.

Methylation. The free acids decomposed readily under rearomatization. They were methylated by addition of small excess of etheral diazomethane.[32] The resulting methylesters were purified and the isomeric products separated by preparative chromatography on silica layers. After being eluted with diisopropylether the pure compounds were crystallized from light petroleum (b.p. 40–60°).

Acetylation. The methylesters were dissolved in 4 ml of pyridine, 1.5 ml Ac_2O added and the resulting solns allowed to stand at room temp. for 18 hr. Brownish oils were obtained after removal of the solvent. The pure 1,2-diacetyl derivatives of the DHB-methylesters were obtained after preparative TLC and recrystallization from methanol–water mixtures.

Preparation of acetonide. The methylester **8** was dissolved in 5 ml of 2,2-dimethoxypropane.[33] The soln was cooled in an ice bath before 0.1 ml of 12N HCl was added. The solvent was removed after standing at room temp. for 7 hr. The resulting crystalline product which was obtained in high yield could be recrystallized from hexane. Calc. mass $C_{12}H_{16}O_4$: 224.1049, Found mass: 224.1055; λ_{max}^{MeOH} 267 nm (ϵ 4,400); λ_{max}^{Nujol} 1,753, 2,900 cm^{-1}; m.p. 47.5–48.5°.

Aromatization of DHB-methylesters. The methylesters were dissolved in MeOH containing 50 μl 12N HCl. The solns of the Me substituted DHBs were allowed to stand at room temp. for 2 hr. The halogen-substituted DHBs were heated to 60° for 24–48 hr. The resulting salicylic acid methylesters were purified on preparative silica layers (solvent system: diisopropylether).

Materials. All chemicals were of the highest purity commercially available. The halo-substituted salicylic acids were synthesized from corresponding aminosalicylic acids by Sandmeyer reaction using the method of Kuhn and Hensel.[34] The methylesters were obtained by methylation with slight excess of etheral diazomethane.

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