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PHENOLIC COMPOUNDS FROM ROOTS OF *URTICA DIOICA*

RUPERT KRAUS and GERHARD SPITELLER

Lehrstuhl Organische Chemie I, Universität Bayreuth Universitätsstraße 30, Postfach 101251, D-8580 Bayreuth, F.R.G.

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Key Word Index—*Urtica dioica*; Urticaceae; stinging nettle; roots; phenolic compounds; lignans; GC-MS.

Abstract—Root extracts from *Urtica dioica* were separated into several classes of compounds by extraction with organic solvents at different pH values. The phenolic fraction was analysed by GC-MS after trimethylsilylation. This procedure allowed the identification of 18 phenolic compounds as well as the detection of eight lignans. The occurrence of some of these substances in this plant was previously unknown.

INTRODUCTION

Extracts from the roots of stinging nettle (*Urtica dioica*) are used for the therapy of prostatahyperplasia. The active principle, however, is still unknown [1]. The aim of our investigation is a thorough inspection of the components present in the roots. Furthermore, we want to check which compounds may be responsible for the described action. In this paper we report on the phenolic components.

In the course of previous investigations of *U. dioica* cinnamic acid derivatives [2], coumarins [1, 3] and homovanillyl alcohol, as well as neoolivil and its acetylated derivatives [4] were detected. These substances were mainly identified by TLC [2, 3] in comparison with reference compounds and by isolation and subsequent identification of the compounds by spectroscopic methods especially NMR [4]. Many of the identified compounds have a 4-hydroxy-3-methoxyphenylpropane structure and are obviously precursors or degradation products of the lignin in the roots.

The isolation of pure samples large enough to run NMR spectra is rather time consuming; components occurring in small amounts are often lost. Much less material is necessary for a GC-MS analysis, which requires the conversion of polar hydroxy groups into trimethylsilyl ethers to enhance volatility and thermal stability. The derivatized samples were subjected to a gas chromatographic separation on capillary columns. Identification was achieved by measuring retention indices and mass spectra with GC-MS. The mass spectra were compared with those of reference compounds which were either commercially available or otherwise synthesized as described in Experimental.

RESULTS AND DISCUSSION

The phenolic fraction from roots of *U. dioica* was obtained in the following way. A concentrated methanol extract was distributed between water and ethyl acetate. Organic acids were removed from the organic layer by extraction with sodium hydrogen carbonate solution and then phenolic compounds were extracted with 2% potassium hydroxide solution. A separation of these very polar compounds by GC required derivatization with MSTFA (*N*-methyl-*N*-trimethylsilyltrifluoroacetamide). This fast and easy derivatization method converts all hydroxy groups into the corresponding trimethylsilyl ethers. Besides hydroxy compounds aldehydes and ketones are also attacked by MSTFA. The basic character of MSTFA causes enolization of ketones which are converted to the trimethylsilyl enol ethers [5]. Aldehydes form addition products with MSTFA [6].

Alternatively diazomethane was used for derivatization, in methanolic solution transforming only phenolic hydroxy groups into the corresponding methyl ethers, while alcoholic hydroxy groups do not react. It is possible to trimethylsilylate the latter groups afterwards. A comparison of the mass spectrometric fragmentation pattern of exclusively trimethylsilylated derivatives and those obtained after methylation/trimethylsilylation provides additional structural information. The information is enhanced if another sample is treated with diazomethane in the presence of silica gel, when methylation of both phenolic and alcoholic hydroxy groups is achieved [7].

Trimethylsilylation and mixed derivatization with diazomethane/MSTFA enabled us to determine the M_r and the number of phenolic hydroxy groups in the phenolic substances of *U. dioica*. Many compounds in the mixture turned out to be close related to one another. This was indicated by key fragments which allowed the deduction of certain structural features (Scheme 1).

Typical fragmentation reactions of aliphatic alcohols, e.g. loss of one or two molecules of $(Me)_3SiOH$ are often observed, while a primary CH_2OTMSi group is indicated by a fragment of m/z 103 ($CH_2=\overset{+}{O}TMSi$) and a secondary hydroxy group by a fragment of m/z 117

The MSTFA adducts of the aldehydes **13** and **16** give characteristic mass spectra [6]. These adducts form slowly if the samples are exposed to MSTFA for several hours. Therefore, these compounds are not found if the reaction time is too short and even after a prolonged reaction period not all aldehyde molecules were converted into derivatives (see Table 1).

Table 1. Chromatographic and mass spectral data of compounds detected in *Urtica* roots

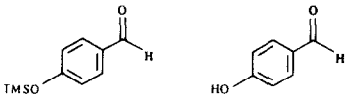
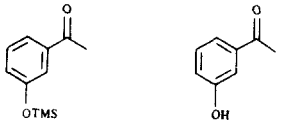
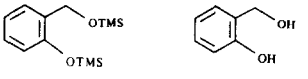
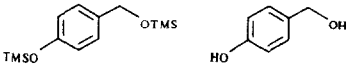
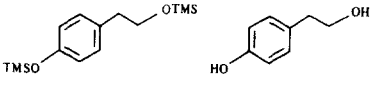
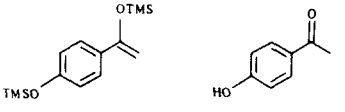
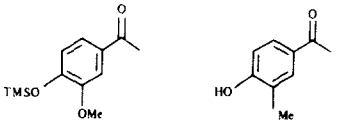
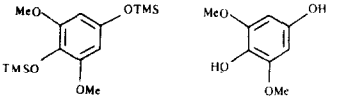
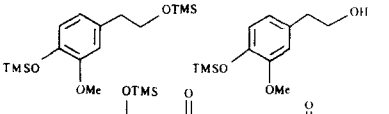
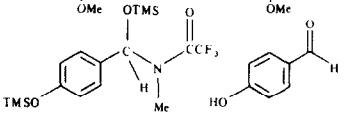
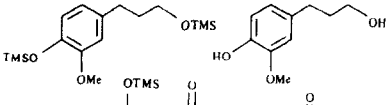
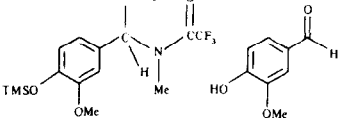
Peak no	Retention index	Identity	Original form	MS *m/z* (% rel. int.)	Ref.
1	1339			195 (10), *194* (66), 193 (3), 181 (5), 180 (14), 179 (100), 152 (3), 151 (34), 135 (6), 91 (8), 89 (16), 77 (6), 75 (12), 73 (16), 58 (6), 45 (16).	[13]
2	1385			209 (8), *208* (58), 194 (16), 193 (100), 165 (12), 151 (18), 147 (14), 135 (4), 91 (8), 89 (10), 77 (12), 75 (50), 73 (22), 58 (14), 43 (38).	
3	1431			269 (14), *268* (62), 267 (20), 254 (12), 253 (54), 209 (6), 179 (32), 163 (10), 149 (11), 147 (34), 135 (6), 133 (12), 105 (5), 75 (10), 73 (100), 59 (4), 45 (10).	
4		Unidentified			
5	1499			269 (18), *268* (68), 267 (41), 254 (8), 253 (38), 223 (5), 180 (16), 179 (100), 163 (6), 147 (34), 119 (6), 117 (4), 89 (4), 75 (8), 73 (78), 59 (4), 45 (12).	
6	1565			283 (5), *282* (24), 267 (12), 193 (10), 180 (14), 179 (100), 147 (3), 126 (4), 103 (10), 75 (56), (3), 45 (8).	
7	1588			281 (20), *280* (38), 279 (86), 265 (62), 223 (8), 192 (6), 191 (17), 147 (10), 131 (6), 115 (4), 91 (3), 77 (10), 75 (28), 73 (100), 69 (10), 58 (6), 45 (24).	
8	1595			239 (16), *238* (74), 224 (18), 223 (100), 209 (8), 208 (58), 194 (10), 193 (72), 173 (6), 165 (10), 147 (6), 137 (8), 104 (6), 89 (8), 77 (8), 75 (10), 73 (42), 59 (10), 45 (16).	[12]
9		Unidentified			
10		Unidentified			
11	1676			315 (31), *314* (100), 299 (11), 285 (10), 284 (50), 254 (5), 135 (5), 75 (15), 73 (27).	
12	1700			313 (20), *312* (62), 297 (16), 282 (3), 223 (3), 210 (16), 209 (100), 193 (3), 179 (9), 134 (4), 103 (5), 75 (6), 73 (51), 59 (3), 45 (3).	[4]
13	1720			395 (6), 394 (16), *393* (46), 392 (70), 378 (10), 269 (9), 268 (21), 267 (82), 265 (12), 193 (10), 192 (9), 184 (19), 147 (3), 134 (6), 110 (22), 92 (4), 91 (4), 77 (18), 75 (44), 73 (100), 45 (14), 44 (18).	[6]
14		Unidentified			
15	1811			327 (16), *326* (48), 312 (6), 311 (10), 237 (8), 236 (22), 223 (5), 221 (10), 210 (17), 209 (22), 206 (64), 205 (32), 180 (10), 179 (24), 149 (8), 148 (8), 141 (10), 103 (5), 89 (19), 75 (16), 73 (100), 59 (27), 45 (17).	
16	1822			424 (16), *423* (38), 422 (30), 408 (10), 298 (17), 297 (72), 267 (6), 265 (5), 223 (8), 184 (12), 134 (5), 110 (21), 77 (18), 75 (28), 73 (100).	[6]

Table 1. *Continued*

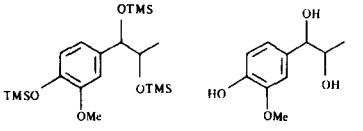
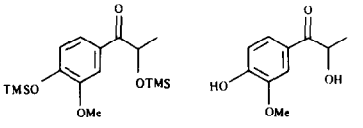
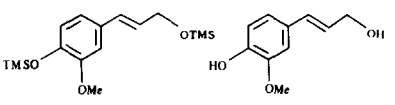
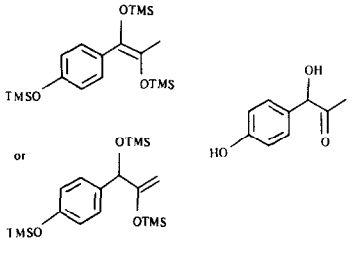
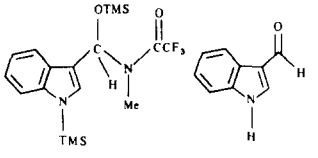
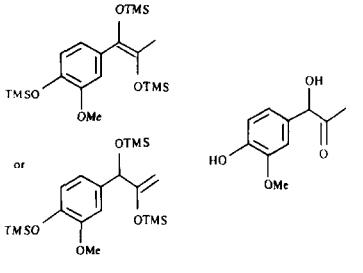
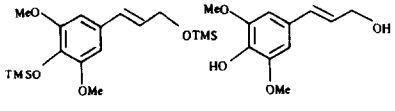
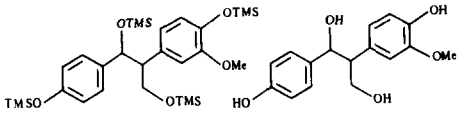
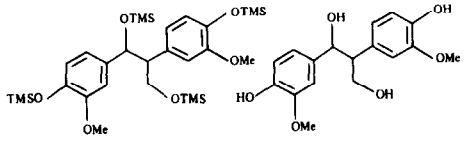
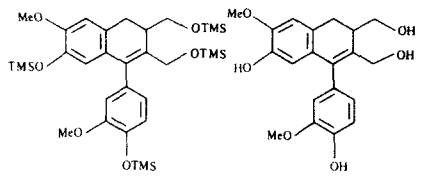
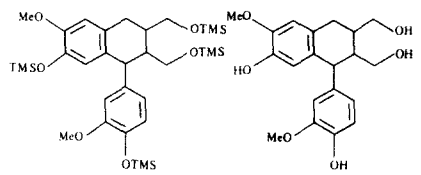
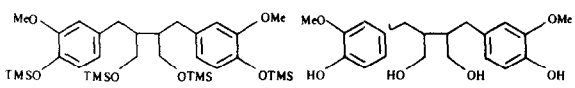
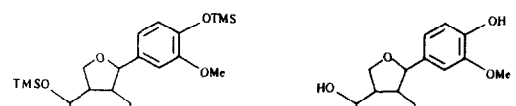
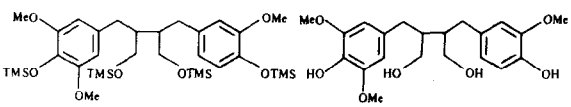
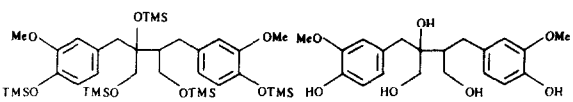
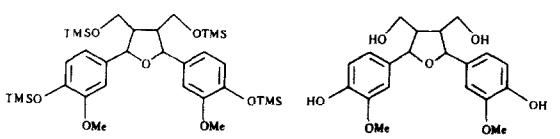
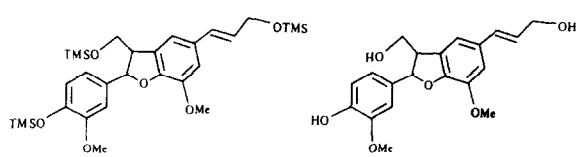
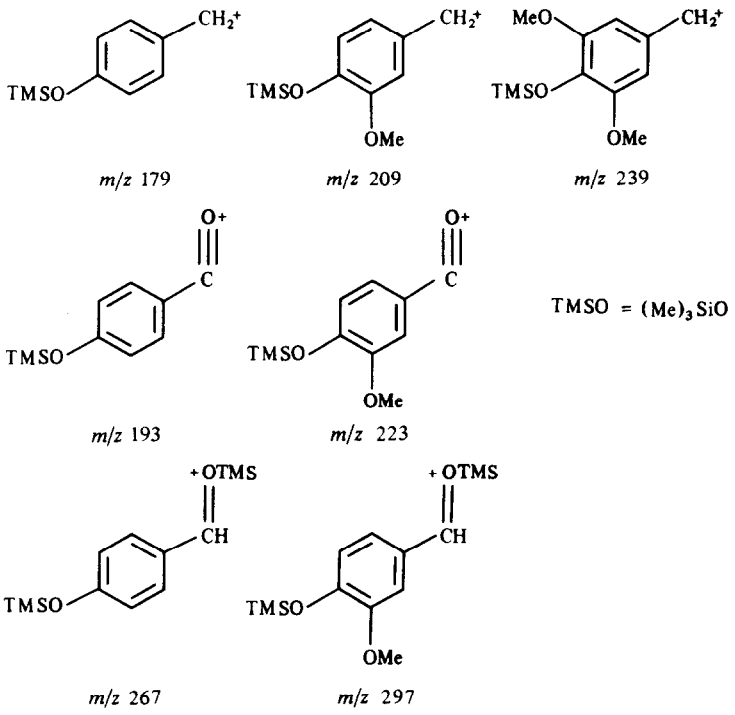
Peak no	Retention index	Identity	Original form	MS *m/z* (% rel. int.)	Ref.
17	1860			400 (3), 399 (7), 299 (17), 298 (4), 297 (100), 267 (5), 223 (3), 194 (3), 147 (9), 117 (4), 75 (9), 73 (68).	
18	1862			*340* (11), 325 (12), 296 (11), 224 (13), 223 (86), 193 (6), 173 (3), 117 (100), 74 (8), 73 (96), 45 (4).	
19	1926			326 (15), 325 (39), *324* (100), 323 (20), 309 (17), 294 (34), 293 (51), 235 (37), 219 (7), 205 (14), 204 (25), 189 (5), 147 (7), 131 (10), 103 (6), 75 (10), 73 (57), 59 (6), 45 (6).	
20	1950			384 (10), 383 (25), *382* (62), 381 (78), 367 (14), 294 (10), 293 (31), 267 (9), 266 (18), 265 (29), 193 (13), 192 (22), 177 (8), 147 (26), 103 (5), 75 (12), 73 (100).	
21	1965			*416* (12), 401 (4), 292 (8), 291 (22), 290 (81), 246 (4), 217 (6), 216 (7), 184 (5), 147 (5), 117 (7), 110 (5), 77 (19), 75 (28), 73 (100), 58 (4), 45 (12).	
22	2070			413 (11), *412* (25), 411 (16), 397 (5), 381 (7), 354 (8), 323 (6), 295 (5), 265 (6), 147 (10), 74 (7), 73 (100), 59 (6), 45 (12).	
23	2074			356 (14), 355 (31), *354* (100), 339 (11), 324 (12), 323 (13), 293 (3), 265 (9), 234 (4), 204 (5), 147 (5), 103 (3), 75 (8), 73 (49), 59 (4), 45 (4).	
24	2543 2554			563 (4), 269 (10), 268 (32), 267 (100), 209 (6), 179 (3), 147 (3), 74 (4), 73 (35).	
25	2607 2623			593 (4), 299 (12), 298 (26), 297 (100), 209 (5), 147 (3), 103 (2), 77 (4), 73 (26).	

Table 1. *Continued*

Peak no	Retention index	Identity	Original form	MS *m/z* (% rel. int.)	Ref.
26				647 (6), *646* (17), 557 (14), 556 (18), 544 (14), 543 (34), 513 (20), 454 (18), 453 (53), 416 (5), 403 (5), 209 (15), 147 (8), 104 (7), 103 (46), 73 (100).	
27				649 (10), *648* (31), 634 (6), 633 (13), 559 (8), 558 (22), 528 (12), 527 (22), 468 (14), 456 (24), 455 (52), 437 (12), 428 (18), 209 (16), 147 (6), 103 (7), 73 (100), 59 (3).	
28				651 (6), *650* (14), 635 (3), 561 (10), 560 (22), 470 (9), 439 (3), 350 (3), 262 (8), 261 (32), 248 (11), 247 (10), 235 (9), 223 (7), 210 (39), 209 (100), 192 (5), 179 (16), 147 (8), 129 (5), 103 (10), 73 (69).	
29				665 (3), *664* (11), 649 (3), 324 (17), 298 (16), 297 (100), 267 (5), 209 (20), 147 (3), 103 (8), 73 (52).	
30				665 (9), *664* (16), 649 (3), 324 (6), 299 (10), 298 (24), 297 (100), 277 (6), 267 (5), 235 (8), 223 (9), 209 (10), 179 (3), 177 (3), 129 (3), 103 (7), 73 (58).	
31				681 (10), *680* (22), 665 (3), 590 (7), 291 (5), 261 (8), 240 (37), 239 (24), 210 (24), 209 (54), 179 (8), 147 (12), 103 (9), 73 (100).	
32				*738* (2), 723 (3), 633 (1), 545 (2), 723 (3), 633 (1), 545 (2), 439 (21), 350 (24), 349 (86), 210 (22), 209 (100), 179 (7), 147 (6), 103 (12), 73 (31).	
33				*664* (4), 649 (7), 574 (3), 341 (22), 340 (78), 325 (23), 324 (51), 251 (12), 250 (37), 247 (18), 235 (8), 223 (23), 209 (14), 193 (5).	[4]
34				575 (12), *574* (27), 559 (4), 544 (10), 486 (14), 485 (39), 484 (100), 471 (5), 454 (4), 394 (4), 310 (3), 209 (11), 103 (15), 73 (98).	

Compound **7** is an example for the formation of trimethylsilyl enol ethers from ketones under basic conditions with MSTFA [5]. The aldehyde **21** was extracted together with the phenolic compounds because of the acidic character of its NH-group. The structures of some lignans (**26**, **29**–**32**) could only be deduced from the mass spectra of the trimethylsilylated and mixed derivatized products (CH_2N_2–MSTFA).

A typical and exemplifying structural identification by various derivatization experiments and mass spectral



Scheme 1. Characteristic mass spectrometric fragment ions of trimethylsilylated phenols.

analysis is described for compound **24**. The mass spectrum of the trimethylsilylated compound showed a base peak at *m/z* 267 which is a typical fragment ion of trimethylsilylated phenols (Scheme 1). Trimethylsilylated compounds often do not show $[M]^+$ ions but $[M-15]^+$ generated by loss of a methyl group. Thus, the M_r of **24** was deduced from the peak at *m/z* 563 to be 578. This was confirmed by treating the original sample with diazomethane/MSTFA to produce **24a**. The mass spectrum of this derivative showed a small but detectable $[M]^+$ at *m/z* 462. In addition the peak of *m/z* 267 was shifted to *m/z* 209 [exchange of one $(Me)_3SiO$ group by a MeO group]. The conversion of two hydroxy groups of the original molecule into methoxy groups by diazomethane (reduction of the M_r in the methylated compound for 116 mass units compared to the trimethylsilylated derivative) indicated the presence of another phenolic hydroxy group in the second part of the molecule.

Considering the phenolic character in this second part of the compound and the characteristic mass differences in both spectra we assumed the presence of a 4-hydroxy-3-methoxy-phenylethanol moiety (D, Scheme 2). The 1,3- or 1,2-diol structure of the two 'aliphatic' hydroxy groups was shown by treatment of the diazomethane-methylated sample with butyldiisopropoxyboronate by formation of a cyclic boronic ester (**24b**). The mass spectrum showed a $[M]^+$ at *m/z* 384 and a base peak at *m/z* 164 which could be attributed to a 3,4-dimethoxystyrene-fragment (E, Scheme 2).

For confirmation of this assumption and in order to distinguish unambiguously between the possible structures A and B (Scheme 2) the sample was converted into a hydrocarbon (**24c**) by treatment of the methylated sample with $MeSO_2Cl$ followed by lithium aluminium hydride reduction (Scheme 3). This method removed the 'aliphatic' hydroxy groups which control the mass spectrometric fragmentation process (especially cleavage at the benzylic hydroxy group).

The resulting 'alkane' fragments of compound **24c** derived by both benzylic cleavage reactions were expected to be produced with about the same probability. The mass spectrum clearly revealed the presence of the structural element C (Scheme 2) because fragment ions for both parts of the molecule are observed as predicted.

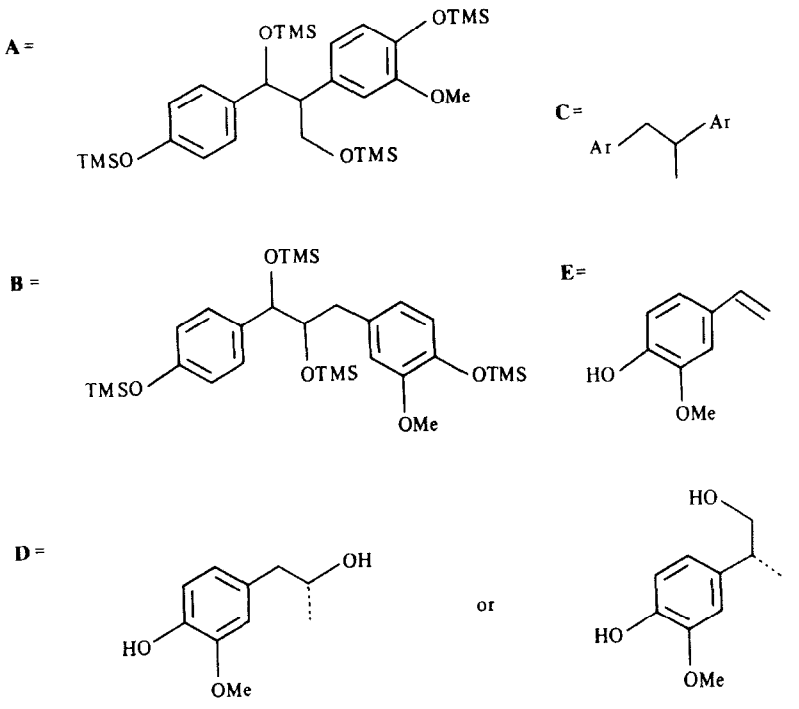
Thus, structure A (Scheme 2) was deduced as the original structure of **24**. This compound is a molecule with two chiral centres. Thus, we can expect two peaks of diastereomers in the GC. In fact there are two peaks at RI 2543 and RI 2554 for compound **24**. The deduction of the absolute configuration of **24** is not possible from the almost identical mass spectra of the isomers and their RI values alone. To our knowledge compound **24** is not yet described in the literature.

EXPERIMENTAL

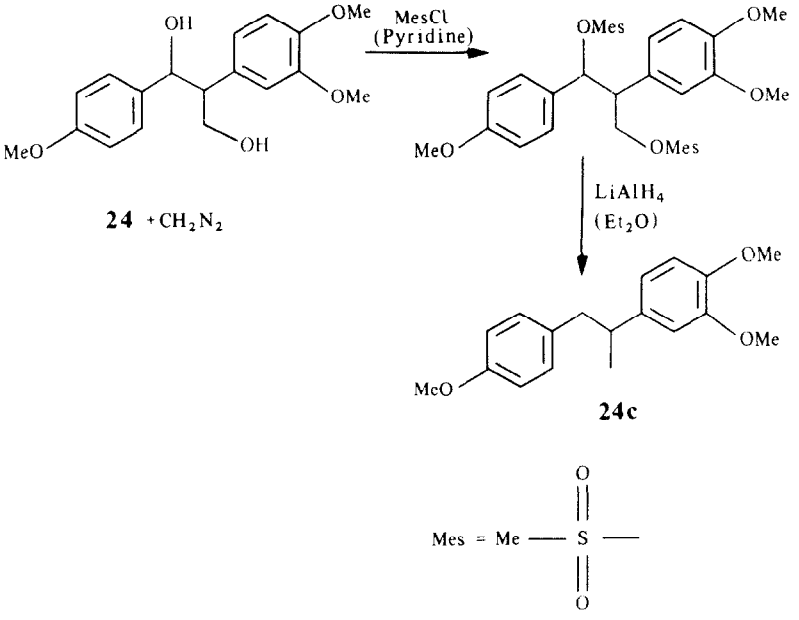
GC-MS was performed on a WCOT-glass capillary column coated with OV-101 (length: 25 m; diameter: 0.3 mm; carrier gas: He 2 ml/min, temp. prog.: 80–280° at 2°/min). It was coupled to a double focusing mass spectrometer running under EI conditions at 70 eV.

For analytical GC the conditions were the same except that the carrier gas was H_2 and the temp. prog. was 80–280° at 3°/min. Retention indices were calculated according to Kováts [8] with *n*-alkanes C_{10}–C_{26} as ref. compounds.

Extraction and separation of compounds. A MeOH–H_2O extract from roots of *U. dioica* concd to a syrupy consistence containing *ca* 20% MeOH was obtained from KANOLDT



Scheme 2. Partial structures of compounds **24**–**24c**.



Scheme 3. Reduction of compound **24** to **24c**.

(8884 Höchstädt, F.R.G.) and used for our investigations. The extract (50 g) was suspended in 200 ml MeOH and 20 g Na_2SO_4 added. The suspension was shaken vigorously for 10 min. The ppt. was removed by centrifugation at 3000 rpm for 10 min. The MeOH soln was concd to *ca* 1/3 of its original vol. and *ca* 200 ml of dil H_2SO_4 added. The mixt. was thoroughly extracted × 4 with 50 ml cyclohexane–Et_2O (2:1) in order to remove nonpolar compounds and then extracted × 4 with 50 ml EtOAc. The combined EtOAc layers were twice washed with a small amount of H_2O. Acids were removed from this layer by extraction × 3 with 3% $NaHCO_3$ soln. After washing with H_2O, the EtOAc layer was evapd to dryness *in vacuo* at 30°, 100 ml aq. 2% KOH soln and 100 ml Et_2O were added. The mixt. was shaken well and the layers sepd immediately. The brown coloured aq. layer

was quickly washed with Et_2O and acidified with 5% HCl. Phenolic compounds were then extracted with EtOAc and the organic layer dried over Na_2SO_4 after washing with H_2O. Evapn to dryness afforded *ca* 50 mg of phenolic compounds.

Trimethylsilylation. Derivatization of phenolic and alcoholic hydroxy groups: 0.3 mg of each substance or mixt. were dissolved in 10 μl THF (purified and dried) and 20 μl MSTFA added. The mixt. was allowed to stand at room temp. for 12 hr or heated in a vial for 30 min at 60°.

Methylation of phenolic hydroxy groups. A MeOH soln of phenols was treated with excess CH_2N_2 in Et_2O for *ca* 5 hr. Excess CH_2N_2 was removed in a stream of N_2.

Methylation of all hydroxy groups [7]. The crude extract (1 mg) was dissolved in EtOAc and 100 mg silica gel 60 (Merck) added. After evapn to dryness the residue was suspended in a small amount of Et_2O and excess CH_2N_2 in Et_2O carefully added. The mixt. was stirred for 24 hr at room temp. with occasional addition of further CH_2N_2. Finally excess CH_2N_2 was removed under a stream of N_2 and the residue extracted with EtOAc.

Identification of compounds. Compounds **1**, **2**, **7**, **8**, **13**, **16** and **21** were commercially available in their native form. After treatment with MSTFA for 12 hr at room temp. they were subjected to GC and GC-MS. R_ts and MS were measured and compared with the data obtained from the extraction sample. The phenolic alcohols **3**, **5**, **6**, **15**, **19** and **23** were synthesized from the Me esters of the corresponding acids by reduction with excess $LiAlH_4$ in Et_2O. Acids were esterified in the usual way with absolute MeOH by addition of catalytic amounts of H_2SO_4 (98%).

Compound **12** (homovanillyl alcohol) was known from the lit. and identified from its MS. Small amounts of compounds **17**, **18** and **22** were obtained by oxidation of *iso*eugenol with 30% H_2O_2–HOAc in 50% aq. EtOH soln for 5 hr at room temp. The resulting mixture contained mainly vanillin, vanillic acid and the above mentioned compounds [9].

For compounds **24** and **25** conversion to the alkane structure was performed as follows. After methylation of the phenolic hydroxy groups with CH_2N_2, compound **24** was enriched from the mixt. by prep. TLC on silica gel with EtOAc–cyclohexane (1:1). Each zone was checked by GC after elution (EtOAc) and trimethylsilylation of the compounds. The zone which contained **24** in the methylated form was treated with $MeSO_2Cl$ in dry pyridine for 2 hr at room temp. Pyridine was completely removed by a stream of N_2 at room temp. and the residue extd thoroughly with dry Et_2O. After addition of fine by powdered $LiAlH_4$ the soln was stirred for 12 hr at room temp. After hydrolysis with ice the Et_2O layer was dried (Na_2SO_4) for several hr and the solvent evapd. The sample of **24c** contained some byproducts as shown by GC.

Lignans **27** and **28** [(+)-isolariciresinol, (−)-secoisolariciresinol] were identified with the aid of comparison samples, a generous gift from Prof. Dr K. Weinges (Heidelberg), after appropriate derivatization (MSTFA) by coinjection and measuring of MS data. Compound **28** and **33** [(−)-secoisolariciresinol, neoolivil] were isolated by prep. TLC and characterized further by ^1H NMR spectrometry. The data were in agreement with the lit. [4, 10]. The structure of the lignans **26**, **29** and **32** could be derived from the typical fragment ions of their trimethylsilyl derivatives and by mixed derivatization with CH_2N_2–MSTFA. Due to the lack of ref. compounds the determination of the absolute configuration was not possible.

Compounds **29** and **33** were found during the dimerization of synthetic coniferyl alcohol with H_2O_2–peroxidase in a buffer solution at pH 6 [11]. Mass spectral data of the trimethylsilyl derivatives are given in Table 1.

Mass spectra of compounds **24a**–**24c**. Compound **24**: EIMS 70 eV (GC-MS) *m/z* (% rel. int.): (see Table 1). Compound **24a**: (same cond.): 462 $[M]^+$ ($C_{24}H_{38}O_5Si_2$) (< 1), 357 $[M-105]^+$ (1), 209 $[C_{11}H_{17}O_2Si_2]$ (100), 151 (3), 121 (3), 73 $[C_3H_9Si]^+$ (52), 45 (1). Compound **24b**: (same cond.): 384 $[M]^+$ ($C_{22}H_{29}O_5B$) (92), 327 (4), 270 (5), 192 (6), 165 (98), 164 $[C_{10}H_{12}O_2]^+$ (100), 149 (49), 135 (16), 121 (43), 91 (16), 77 (19). Compound **24c**: (same cond.): 286 $[M]^+$ ($C_{18}H_{22}O_3$) (14), 165 $[C_{10}H_{13}O_2]^+$ (100), 150 (4), 134 (3), 121 $[C_8H_9O]^+$ (15), 105 (2), 78 (2), 77 (2), 65 (1).

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