Phytochemistry, Vol 23, No 10, pp 2199–2201, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00
Pergamon Press Ltd

ODORATOL AND METHYLODORATOL, TWO α-HYDROXYDIHYDROCHALCONE STRESS METABOLITES FROM *LATHYRUS ODORATUS*

A FUCHS, F W DE VRIES, C A LANDHEER* and A VAN VELDHUIZEN*

Laboratory of Phytopathology, Agricultural University, Wageningen, The Netherlands, *Laboratory of Organic Chemistry, Agricultural University, Wageningen, The Netherlands

(*Received* 3 *February* 1984)

Key Word Index—*Lathyrus odoratus*, Vicieae, Leguminosae, sweet pea, phytoalexins, stress metabolites, flavonoids, α,2′-dihydroxy-4′,4-dimethoxydihydrochalcone, odoratol, α-hydroxy-2′,4′,4-trimethoxydihydrochalcone, methylodoratol, fungitoxicity

Abstract—From *Lathyrus odoratus* treated with mercuric acetate (cotyledons, pods) or with *Phytophthora megasperma* var *sojae*-elicitor (cotyledons) a number of fungitoxic stress metabolites have been isolated, among which are two novel α-hydroxydihydrochalcones (odoratol and methylodoratol) Their structures have been elucidated

INTRODUCTION

Many species of the genus *Lathyrus* produce pterocarpan phytoalexins upon inoculation with a spore suspension of *Helminthosporium carbonum* [1] Among these pisatin is the major one [2], followed by maackiain, variabilin and medicarpin *L nissolia* is the only species which, besides medicarpin, produces nissolin and methylnissolin [3], whereas upon inoculation of *L odoratus* and *L hirsutus* with either *Botrytis cinerea* or *H carbonum*, together with pisatin and variabilin, two chemically unrelated phytoalexins are formed, viz the chromones lathodoratin and methyl-lathodoratin [4]

TLC bioassays, using *Cladosporium cucumerinum* as the test fungus [5], of petroleum ether and ethyl acetate extracts of mercuric acetate-treated pods and mercuric acetate or Pms-elicitor-treated (see Experimental) cotyledons of *L odoratus* revealed a number of inhibition zones The compounds involved appeared to be the pterocarpan pisatin and the chromones lathodoratin and methyl-lathodoratin, a fourth inhibition zone was due to the presence of a strongly lipophilic compound, 2-(2-cyanoethyl)isoxazolin-5-one [6], which was also found in extracts of non-treated pods of *L odoratus* In addition, two compounds were detected which closely resembled each other Their chemical characterization as α-hydroxydihydrochalcones is reported in the present paper This is the first report of this class of flavonoids within the tribe Vicieae in the family Leguminosae, the presence of two *C*-glucosylated α-hydroxydihydrochalcones in the trunk wood of *Eysenhardtia polystachya* (tribe Amorpheae [7]) has been reported recently [8]

REFULTS AND DISCUSSION

Of six reagents for flavonoids and isoflavonoids tested [saturated aqueous lead(II) acetate, diazotized *p*-nitroaniline (cf [9]), dinitrophenyl-hydrazine (cf [9]), 1% aqueous ferric chloride, Gibbs reagent (2,6-dichloroquinone-4-chloroimide, followed by aqueous sodium carbonate, cf [9]), 1% aqueous sodium carbonate] only Gibbs reagent afforded a blue product with the minor compound (**1**), whereas with the predominant one (**2**) a brownish blue colour was obtained, with 1% aqueous sodium carbonate the latter compound turned faintly yellow

The UV spectrum of this compound [λ_{max}^{EtOH} nm 226 (100%), 272 (62%), 305 (40%)] strongly resembled those of the flavanones liquiritigenin (λ_{max}^{EtOH} nm 234, 277, 312) and butin (λ_{max}^{EtOH} nm 233, 278, 312, cf [10]) The latter compounds readily undergo conversion into their corresponding chalcones, producing equilibrium mixtures of liquiritigenin and isoliquiritigenin (2′,4′,4-trihydroxychalcone) and butin and butein (2′,4′,3,4-tetrahydroxychalcone), respectively (cf [11]) These chalcones are the most common among the thirteen listed by Wollenweber and Dietz [12] as occurring in the Leguminosae

The elemental composition of the molecular ion (*m/z* 316) of **2** ($C_{18}H_{20}O_5$) precluded its classification as a chalcone However, presence of a carbonyl function (ν_{max} 1655 cm^{-1}) and thermal loss of one molecule of water at sample temperatures > 120° suggested it to be an α or β-hydroxydihydrochalcone The NMR data indicate the presence of three methoxyl groups distributed over two benzene rings Considering the aromatic patterns and comparing our data with those on the related compound nubigenol [13] identification of **2** as a dihydrochalcone structure with three methoxyl groups at positions 2′, 4′ and 4 seems most plausible

The base peak in the mass spectrum at *m/z* 165 and the elemental composition of the fragment ion involved ($C_9H_9O_3$, Table 1), suggest the disubstituted aromatic ring to be linked to the carbonyl group of the chalcone This ion can be assumed to be formed by a simple cleavage of the molecular ion between the carbonyl group and the α carbon atom Fragments with masses 195 and 121 strongly suggest a simple fragmentation of the molecular ion to take place between the α and the β carbon atom, with the hydroxyl group at the α position Both fragments are present in the mass spectrum as intense peaks, and their accurate masses and elemental composition (Table 1) are in accordance with the proposed structure for **2** (Fig 1, R = Me) A similar cleavage with the

Table 1 EI mass spectral data of 1 and 2 at a sample temperature of 120°

m/z	Rel int	Exp mass	Elem comp	Calc mass
Odoratol (1)*				
302	3 5			
284	20	284 1036	$C_{17}H_{16}O_4$	284 1049
181	6 5			
151	19 5	151 0393	$C_8H_7O_3$	151 0395
121	100	121 0657	C_8H_9O	121 0653
Methylodoratol (2)†				
316	2 3	316 1312	$C_{18}H_{20}O_5$	316 1311
298	23	298 1205	$C_{18}H_{18}O_4$	298 1205
195	23	195 0654	$C_{10}H_{11}O_4$	195 0657
167	84	167 0715	$C_9H_{11}O_3$	167 0708
165	100	165 0552	$C_9H_9O_3$	165 0552
151	5			
122	17			
121	44	121 0652	C_8H_9O	121 0653

*A metastable peak is present at m/z 267 1

†The fragment peaks given all result from primary cleavages of the molecular ion 316^+, as is indicated by metastable B/E and HV scans

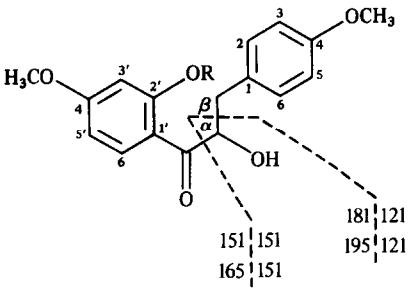

Fig 1 Structural formulae of odoratol (R = H) and methylodoratol (R = Me), dashed lines indicate fragmentation pattern (top odoratol fragment ions, bottom methylodoratol fragment ions)

hydroxyl group at the β position would give rise to two other fragments 179 and 137 Fragment 179^+ is completely absent in the mass spectrum, whereas 137^+ is present only as a minor peak, which could have been formed by loss of CO from the fragment ion m/z 165

Compound 1 is closely related and its structure can be deduced in an analogous way Here, the NMR data suggest a dihydrochalcone structure with two methoxyl groups and one aromatic hydroxyl group From substituent effect calculations it is reasonable to suppose that the methoxyl groups are at positions 4' and 4, and the hydroxyl group thus at position 2' The structure proposed in Fig 1 (R=H) is in accordance with the mass spectral data of 1 (Table 1) In this case no accurate mass measurements have been made of the molecular ion peak at m/z 302 However, the fragment ion m/z 284 is due to loss of water, as supported by the presence of a metastable peak at m/z 267 1 This observation is in agreement with the mass spectral behaviour of 2 (Table 1) From the elemental composition of the fragment ion m/z 284 ($C_{17}H_{16}O_4$, Table 1), it can be concluded that the elemental composition of 1 is $C_{17}H_{18}O_5$ For 1 the name odoratol is proposed, its methyl ether 2 can thus be named methylodoratol

Since odoratol and methylodoratol are produced as a result of both treatment with mercuric acetate and application of Pms-elicitor [14] they can be considered as stress metabolites Since both are induced fungitoxins, they can also be classified as phytoalexins With their identification, two novel phytoalexins can now be added to the already long list of *L odoratus* phytoalexins Since neither the chromones lathodoratin and methyllathodoratin nor the α-hydroxydihydrochalcones (nor the isoxazolin-5-one derivatives) have, so far, been found in other species except for the chromones in the closely related species *L hirsutus*, the taxonomic position of these species within the genus *Lathyrus* is a very isolated one (cf [1, 15])

α-Hydroxydihydrochalcones are very rare in nature so far, they have only been found—free or in *C*-glucosidic form—in three unrelated species, viz *Podocarpus nubigena* (Podocarpaceae, leaves and stems) [13], *Lyonia formosa* (Ericaceae, leaves) [16] and *Eysenhardtia polystachya* (Leguminosae, trunk wood) [8] This is the first report of α-hydroxydihydrochalcones as phytoalexins or stress compounds in the tribe Vicieae Only very recently [17] *Pisum sativum*, another species in the Vicieae, has been shown to produce a 2'-methoxychalcone as a stress metabolite

EXPERIMENTAL

Pods of *Lathyrus odoratus* L were obtained from the Botanical Garden of the Delft University of Technology and from the Demonstration Garden for Plant Diseases of the Laboratory of Phytopathology, Agricultural University, Wageningen After being collected the pods were stored at 4° for 3 to 4 weeks to maximize subsequent phytoalexin (stress compound) production [18] Cotyledons were obtained from 1-week-old seedlings In pod halves as well as cotyledons production of phytoalexins was induced by a cell-wall preparation of *Phytophthora megasperma* var *sojae* (Pms-elicitor [14]), using the drop diffusate technique (cf [9]), or by immersing them in an aerated Hg-acetate-containing mineral soln, which was refreshed daily for 3 to 4 days Drops and aqueous solns were collected and the solutes partitioned into petrol (bp 40–60°) or EtOAc After being dried and taken up in small amounts of EtOH, both petrol and EtOAc extractives were subjected to TLC on Al sheets precoated with silica gel 60 F_{254} (Merck) in $CHCl_3$–MeOH (97 3) When viewed in UV light, just in front of pisatin (R_f 0 71) there was a fluorescence-quenching dark area, with R_f 0 82, which upon TLC in *n*-hexane–EtOAc–MeOH (60 40 1) was resolved into two dark zones with R_f 0 55 (1) and 0 40 (2), respectively After spraying the TLC plates with a conidial suspension of *Cladosporium cucumerinum* [5] and incubating for 2 days at 23°, inhibition zones proved the compounds to be fungitoxic Purification was achieved using repeated TLC with *n*-hexane–EtOAc–MeOH (60 40 1)

^{1}H NMR spectra were recorded on a Bruker CXP-300 spectrometer using Quadrature detection with 16 K data (Hz/Pt = 0 37) and a spectral width of 3000 Hz $CDCl_3$ was used as solvent, with TMS as internal standard

α,2'-Dihydroxy-4',4-dimethoxydihydrochalcone (1, odoratol) UV λ_{max}^{EtOH} nm 222 (100%), 278 (53%), 315 (25%) (for full spectral data, see ref [15]) MS see Table 1 ^{1}H NMR ($CDCl_3$) δ2 91

(*dd*, J = 6 5 and 14 Hz, H β pos), 3 14 (*dd*, J = 3 5 and 14 Hz, H β pos), 3 51 (*d*, J = 8 0 Hz, OH α pos), 3 78 and 3 88 (*s*, 2 OMe groups), 5 21 (*m*, H α pos), 6 48 and 6 50 (*m*, H-3′ and H-5′), 6 81 (*d*, J = 8 5 Hz, H-3 and H-5), 7 06 (*d*, J = 8 5 Hz, H-2 and H-6), 7 59 (*d*, J = 9 0 Hz, H-6′), 12 1 (*s*, OH phenolic)

α-Hydroxy-2′,4′,4-trimethoxydihydrochalcone (**2**, methylodoratol) UV λ_{max}^{EtOH} nm 226 (100%), 272 (62%), 305 (40%) (for full spectral data, see ref [15]) IR ($CHCl_3$) 3450, 1655 and 1600 cm^{-1} MS see Table 1 ^{1}H NMR ($CDCl_3$) δ 2 64 (*dd*, J = 7 5 and 14 Hz, H β pos), 3 07 (*dd*, J = 3 5 and 14 Hz, H β pos), 3 78, 3 90 and 3 94 (*s*, 3 OMe-groups), 5 30 (*m*, H α pos), 6 49 (*d*, J = 2 5 Hz, H-3′), 6 61 (*dd*, J = 2 5 and 9 0 Hz, H-5′), 6 80 (*d*, J = 8 5 Hz, H-3 and H-5), 7 08 (*d*, J = 8 5 Hz, H-2 and H-6), 7 90 (*d*, J = 9 0 Hz, H-6′)

Acknowledgements—The authors are indebted to Mr W P Combé, Laboratory of Organic Chemistry, Agricultural University, Wageningen, for skilful MS analyses, and to Dr P J W Schuyl, Ir A Sinnema and Mr J M van der Toorn, Department of Organic Chemistry, Delft University of Technology, Delft, for confirming the MS and NMR data

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