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β-DIKETONES IN *RHODODENDRON* WAXES

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Abstract—Chromatographic, mass spectrometric and spectroscopic evidence has been obtained for four β-diketones occurring in the leaf waxes of some members of the genus *Rhododendron*. The commonest compound is nonacosane-8,10-dione (confirmed in 22 species and suspected in 18 others). Nonacosane-12,14-dione was detected in large amounts only in *R. campylogynum* whilst hentriacontane-14,16-dione was confirmed as the major β-diketone (along with a small amount of nonacosane-8,10-dione) and as a major surface lipid in 5 species. Hentriacontane-10,12-dione was found in four species.

INTRODUCTION

The investigation of the possible utilisation of chemical taxonomy, using leaf-surface waxes, has been initiated as part of a programme for the reclassification of the genus *Rhododendron* in collaboration with the Royal Botanic Garden, Edinburgh. A number of unusual substances having apparent significance for the classification of this genus have been detected by GLC and GC–MS. This paper describes the identification and distribution of four long-chain β-diketones. Table 1 lists the various β-diketones which have been reported previously in plant waxes [1–16].

RESULTS AND DISCUSSION

Table 2 lists the properties of β-diketones isolated from waxes of *Rhododendron* leaves and Table 3 those species examined in which one or more β-diketone could be demonstrated by GC-MS analysis. The quantitative estimates are very approximate and should only be taken as a guide to the best sources of these compounds. Other components of these leaf waxes include hydrocarbons, diterpenes, triterpenes (e.g. campanulin, friedelin, α- and β-amyrin), aldehydes, alcohols and fatty acid methyl esters.

The UV and IR spectra observed for the com-

Table 1. β-Diketones isolated from plant waxes

Nonacosane-6,8-dione $[Me(CH_2)_4COCH_2CO(CH_2)_{20}Me]$	Small amounts in *Buxus sempervirens* [13]
Nonacosane-12,14-dione $[Me(CH_2)_{10}COCH_2CO(CH_2)_{14}Me]$	Small amounts in many *Eucalyptus* species [2]
Hentriacontane-8,10-dione $[Me(CH_2)_6COCH_2CO(CH_2)_{20}Me]$	*Buxus sempervirens* [13]
Hentriacontane-12,14-dione $[Me(CH_2)_{10}COCH_2CO(CH_2)_{16}Me]$	Several species [1]
Hentriacontane-14,16-dione $[Me(CH_2)_{12}COCH_2CO(CH_2)_{14}Me]$	Small amounts in many *Eucalyptus* species [1,2] *Triticum* species [5–9, 11, 12] Barley [9, 10] *Secale cereale* [14, 15] *Puccinia striiformis* [16]
Tritriacontane-10,12-dione $[Me(CH_2)_8COCH_2CO(CH_2)_{20}Me]$	Small amount in *B. sempervirens* [13]
Tritriacontane-16,18-dione $[Me(CH_2)_{14}COCH_2CO(CH_2)_{14}Me]$	Many *Eucalyptus* species [2]

Table 2. Data for β-diketones isolated from *Rhododendron* leaf

UV	max	274 nm	
IR	cm^{-1}	1730, 1700, 1640–1610, 1475, 1420, 905, 819, 785, 770, 722, 718.	
GLC	RI_{290}^{SE-33}	Compounds 1 and 2: 3190 Compounds 3 and 4: 3390	
	RI^{OV-17}	Compounds 1 and 2: 3310 Compounds 3 and 4: 3510	
GC–MS	Compound 1 Nonacosane-8,10-dione	*m/e*	436, 418, 365, 352, 337, 334, 295, 276, 184, 169, 166, 127, 108, 100.
	Compound 2 Nonacosane-12,14-dione	*m/e*	436, 418, 309, 296, 281, 278, 253, 240, 239, 225, 222, 220, 183, 164, 100.
	Compound 3 Hentriacontane-14,16-dione	*m/e*	464, 446*, 309†, 296‡, 281, 278, 268, 253, 250, 239, 220§, 211, 192‖, 100¶.
	Compound 4 Hentriacontane-10,12-dione	*m/e*	464, 446, 365, 352, 337, 334, 295, 276, 212, 197, 194, 155, 136, 100.

* 446·4484; $C_{31}H_{50}O$ requires 446·4487.
† 309·2796; $C_{20}H_{37}O_2$ requires 309·2793.
‡ 296·2708; $C_{19}H_{36}O_2$ requires 296·2715.
§ 220·2192; $C_{16}H_{28}$ requires 220·2191.
‖ 192·1871; $C_{14}H_{24}$ requires 192·1878.
¶ 100·052; $C_5H_8O_2$ requires 100·052.

Table 3. Rhodendron species containing β-diketones as wax components

Subgenus	Species	Code*	β-diketones present (approx. conc. mg/100 g fr. wt.) 1	2	3	4	Unidentified β-diketones chain length C‡
RHODODENDRON							
Section Rhododendron							
Subsection							
Boothia§	*R. leucaspis*	250125	15 (8·5)†	—	—	—	
	R. megeratum	699558	15 (3·5)	—	—	—	
Cinnabarina	*R. cinnabarinum*	380362	70 (34)	—	—	—	(27, 31)
	R. concatenans	250167	35 (41)	—	—	—	(27, 31)
	R. keysii	698677	10 (13)	—	—	—	
Campylogyna	*R. campylogynum*						
	var. campylogynum	699485	—	50 (47)	—	—	
	var. charopoeum	340299	—	300 (70)	—	—	
	var. myrtilloides	699488	—	300 (47)	—	—	
Glaucophylla	*R. glaucophyllum*	698599		⩾ 100(> 50)		—	
Genesteriana	*R. genesterianum*	540075	—	—	250 (> 50)	—	29
Lapponica	*R. fastigiatum*	734283	300 (35)	—	—	—	
Baileya	*R. baileyi*	380593	5 (1·5)	—	—	100 (29)	
Maddenia	*R. crassum*	490184	—	—	—	10	
	R. formosum	730900	2	—	—	—	(27, 31)
	R. iteophyllum	480095	1	—	—	—	
	R. lindleyi	754044	+ ‖	—	—	20	
	R. maddenii	754068	2	—	—	—	
	R. manipurense	754057	100 (14)	—	—	—	(27,31)
Saluenensia	*R. calostrotum*	270015	100 (23)	—	—	—	
	R. saluenense	490189	—	—	—	—	29
Triflora	*R. amesiae*	200006	—	—	—	—	31
	R. bauhiniflorum	280092	—	—	200 (> 50)	—	
	R. concinnum	698482	—	—	40	—	
Subgenus Pseudorhodoras-trum							
Section Pseudorhodoras-trum	*R. virgatum*	699614	25 (29)	—	—	—	
Rhodobotrys	*R. racemosum*	754071	6	—	150 (60)	—	
Trachyrhodion	*R. hemitrichotum*	698631 698632	10		~ 500 (> 60)		

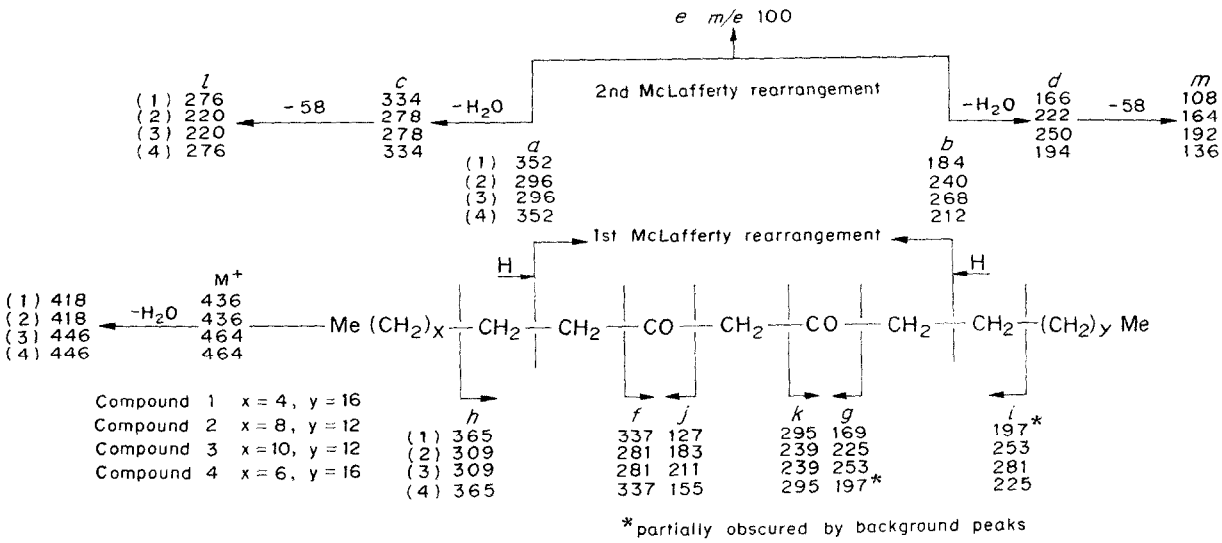
Table 3. (*Continued*)

Subgenus	Species	Code*	β-diketones present (approx. conc. mg/100 g fr. wt.) 1	2	3	4	Unidentified β-diketones chain length C‡
Subgenus AZALEA							
Section Rhodora	*R. canadense*	400066	—	—	—	60	
Subgenus HYMENANTHES							
Section Hymenanthes							
Subsection Barbata	*R. bainbridgeanum*	310497	—	—	—	—	29
	R. smithii	140009	—	—	—	—	29
Campanulata	*R. campanulatum*	550069	—	—	—	—	29
Neriiflora	*R. pocophorum*	290006	—	—	—	—	29
	R. bureavii	180017	—	—	—	—	29
Taliensia	*R. phaeochrysum*	698780	+	—	—	—	29
	R. prattii	698801	—	—	—	—	29
Thomsonia	*R. cerasinum*	754047	+	—	—	—	
	R. eclecteum	230016	—	—	—	—	29
	R. hylaeum	470106	—	—	—	—	(27)29
	R. thomsonii	754074	—	—	—	—	29
Campylocarpa	*R. callimorphum*	698429	—	—	—	—	29 (31)
	R. campylocarpum	754061	+	—	—	—	
Martiniana	*R. martinianum*	754052	+	—	—	—	
Selensia	*R. dasycladum*	330314	—	—	—	—	29
	R. jucundum	698674	+	—	—	—	
	R. rhaibocarpum	734196	—	—	—	—	29
	R. selense	190026	—	—	—	—	29
	R. setiferum	614565	—	—	—	—	29
Soulia	*R. wardii*	190026	—	—	—	—	29

* Accession numbers for plants growing in the Toyal Botanical Garden, Edinburgh from which leaves were harvested.
† Figures in parentheses denote approximate percentage of the total wax analyzable by GLC on OV-17.
‡ Determined by single ion monitoring at *m/e* 100 using GC-MS.
§ Classification according to Sleumer [19] as adapted by Dr J. Cullen, Edinburgh [20].
‖ Denotes compound identified by MS but present only in small amount.

pounds listed in Table 2 resemble those described for acetylacetone and also those for the various β-diketones reported in plant waxes [1–16]. The MS fragmentation of β-diketones have also been described [9, 13, 17] and the MS data in Table 2 are consistent with the previous data and with the structures assigned. The availability of four compounds as two pairs of homologous compounds enabled us to verify the interpretation of the MS as shown in Scheme 1 [17]. The strongest peaks in all spectra were *e* and the α-cleavage monoketone fragments *j* and *k*. The McLafferty rearrangement [18] and dehydration fragments *a-d* have been described previously [9,17] and the peak *e* at *m/e* 100 is presumed to arise by the double rearrangement [17]. This ion (MW 100·052) has the formula $C_5H_8O_2$ and, if sufficiently high resolution MS is used so as to resolve it from the peak at *m/e* 100 arising from $^{13}C_1^{12}C_6H_{15}$ (MW 100·121), serves as a suitable ion for single ion mass fragmentography of these compounds (Table 3). The presence of a long hydrocarbon chain on either side of the β-diketone enables the asymmetry of the substitution to be determined by MS, probably as easily as is the case using alkaline hydrolysis to give the monoketone derivatives [1,2].

All compounds observed in the present work appeared essentially homogenous except that *R. racemosum* and *R. bauhiniflorum* contained about 10% of nonacosane-8,10-dione associated with the hentriacontane-14,16-dione and small amounts of C_{27} and C_{31} compounds could be detected in some extracts. It has been found in other work



[9–12] that the occurrence or absence of β-diketones in wheat and barley could be correlated with single gene mutations, and Tulloch has reported [8] that the distribution of these compounds on the wheat leaf surface is very specific and also age dependent. The role of these compounds in the wettability of wheat leaves has been discussed in detail [12]. Thus the biosynthesis of these compounds appears to be well controlled, a possibility which is also consistent with the high purity of many isolates of β-diketones as compared to other surface lipids and also with the fact that usually when two or more homologous compounds occur the functional group is to be found at a constant distance from one end of the carbon chain (usually the longer chain 'y' is fixed). On this basis it is possible that the *Rhododendron* species examined in this work contain several genes enabling synthesis of β-diketones and that *R. racemosum* and *R. bauhiniflorum*, which contain the genes for synthesis of hentriacontane-14,16-dione, may contain separate genes for synthesis of nonacosane-8,10-dione, since these two compounds are not structurally directly related. It is also possible that one gene selects for carbon chain length (C_{29} or C_{31}) and that another selects for the position in the chain of the β-diketone group. *R. campylogynum* contains a gene combination for synthesis of nonacosane-12,14-dione, a compound which is related to hentriacontane-14,16-dione. However, this species is the only one so far in which this particular isomer of nonacosanedione has been detected in the present work. It appears that nonacosane-8,10-dione (compound 1) is a novel compound of leaf waxes not having been described previously. It is the commonest β-diketone detected in the genus *Rhododendron* having been detected in 22 species out of more than 150 examined. It would appear also that the homologue hentriacontane-10,12-dione is also a novel compound (found in 4 species in the present work).

The taxonomic significance of β-diketones in the genus *Rhododendron* is not clear, as uniform distribution in various subsections was only observed for subsections Boothia and Cinnabarina and the number of species was limited to two and three respectively. It was noticable however that these compounds were prominent (e.g. 8–60%) components of leaf waxes more frequently amongst Lepidote rather than Elepidote species. Amongst Elepidote Rhododendrons (Subgenus Hymenanthes) such compounds are present most frequently amongst the Thomsonia series and only in smaller amounts (<10%). It is interesting to note also that the subsection Maddenia can be divided into three groups: (a) those containing no β-diketones—*R. ciliatum* and *R. fletcherianum*; (b) those containing compound 1—*R. formosum*, *R. iteophyllum*, *R. lindleyi*, *R. maddenii* and *R. manipurense* and (c) those containing compound 4—*R. crassum* and *R. lindleyi*. We believe that an inves-

tigation of hybrids derived from the various species listed in Table 3 would afford much valuable information on the genetic control of the biosynthesis of β-diketones.

EXPERIMENTAL

Rhododendron species were harvested so as to ensure, where possible, that mature leaves (*ca* 1 yr old) were available for extraction. Waxes were obtained by immersing leaves in $CHCl_3$ for 1 min. Extracts were concentrated to a small vol and analysed by GLC after addition of *n*-dotriacontane (1 mg) as internal standard. GLC was performed using 3 m columns packed with either 2% SE-33 or 3% OV-17 on Gas Chrom Q. Operating conditions were, for SE-33 isothermal at 285° and for OV-17, temp. programmed at 4°/min from 190–290° then held at 290° for 20 min. GC–MS analysis was performed using a 6 m column packed with 1% OV 17 and the separator maintained at 250–260°, ion source at 250° with an ionising voltage of 24 eV. High resolution mass determinations were determined using a direct insertion probe. β-Diketones were isolated using PLC on 1 mm thick Si gel G layers with petrol–$CHCl_3$ (7:3). Bands were detected using dichlorofluorescein and the diketones had an R_f of *ca* 0·3–0·4.

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REFERENCES

1. Horn, D. H. S. and Lamberton, J. A. (1962) *Chem. Ind. (London)* 2036.
2. Horn, D. H. S., Kranz, Z. H. and Lamberton, J. A. (1964) *Aust. J. Chem.* **17**, 464.
3. Saayman, H. M. and Roux, D. G. (1965) *Biochem. J.* **97**, 794.
4. Hellyer, R. O. (1968) *Aust. J. Chem.* **21**, 2825.
5. Tulloch, A. P. and Weenink, R. O. (1969) *Can. J. Chem.* **47**, 3118.
6. Tulloch, A. P. and Hoffman, L. L. (1971) *Phytochemistry* **10**, 871.
7. Tulloch, A. P. and Hoffman, L. L. (1973) *Phytochemistry* **12**, 2217.
8. Tulloch, A. P. (1973) *Phytochemistry* **12**, 2225.
9. Jackson, L. L. (1971) *Phytochemistry* **10**, 487.
10. Von Wettstein-Knowles, P. (1972) *Planta* **106**, 113.
11. Barber, H. N. and Netting, A. G. (1968) *Phytochemistry* **7**, 2089.
12. Netting, A. G. and Von Wettstein-Knowles, P. (1973) *Planta*, **114**, 289.
13. Dierickx, P. J. (1973) *Phytochemistry* **12**, 1498.
14. Dierickx, P., and Compernolle, F. (1974) *Phytochemistry* **13**, 682.
15. Streibl, M., Konečný, K., Trka, A., Ubick, K. and Pazlar, M. (1974) *Coll. Czech. Chem. Commun.* **39**, 475.
16. Jackson, L. L., Dobbs, L., Hildebrand, A. and Yokiel, F. A. (1973) *Phytochemistry* **12**, 2233.
17. Trka, A. and Streibl, M. (1974) *Coll. Czech. Chem. Commun.* **39**, 468.
18. Gilpin, J. A. and McLafferty, F. W. (1957) *Analyt. Chem.* **29**, 990.
19. Sleumer, H. (1949) *Bot. Jahrb.* **74**, 512.
20. Cullen, J. Personal communication.