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HOMOLOGOUS LONG-CHAIN ALKANEDIOLS FROM *PAPAVER* LEAF CUTICULAR WAXES

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Key Word Index—*Papaver*; Papaveraceae; cuticular wax; leaves; alkanediols; biosynthesis.

Abstract—In cuticular waxes from leaves of *Papaver alpinum* sensu Markgr., *P. dubium* L., *P. nudicaule* L., *P. orientale* L., *P. rhoeas* L. and *P. somniferum* L., six new alkanediols carrying one primary and one secondary hydroxyl function were identified. Five of them, docosane-1,3-diol, hexacosane-1,7-diol, octacosane-1,9-diol, nonacosane-1,10-diol and triacontane-1,11-diol, form a homologous series. Their molecular geometries and relative proportions suggest a biosynthetic relationship to the corresponding primary alkanols.

INTRODUCTION

Recently, the chemical composition of cuticular waxes from leaves and fruit capsules of eight *Papaver* species has been investigated [1]. Wax mixtures were dominated by secondary alkanols, mainly nonacosan-10-ol. In addition, substantial amounts of secondary alkanediols, e.g. nonacosane-5,10-diol and nonacosane-7,10-diol, were detected. All the prevailing (non-vicinal) diol isomers share the nonacosane carbon backbone and carry one hydroxyl group in the 10-position. Therefore, it seems probable that nonacosan-10-ol is the precursor for the biosynthesis of respective alkanediols [2]. By comparing the isomer compositions of the two wax fractions, both their biosynthetic relationship and the specificity of the involved reactions could be deduced [1].

In order to test this approach, additional classes of isomer-containing wax constituents had to be investigated. Therefore, we re-examined some of the minor fractions of *Papaver* waxes that had not been identified before. Improved spectral data and the synthesis of an authentic standard now led to the identification of six compounds in a previously unknown homologous series of alkanediols carrying one primary and one secondary hydroxyl group. Their isomer composition can supply information on their biosynthesis, e.g. on the sequence of the two hydroxylation steps involved.

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RESULTS AND DISCUSSION

Within the fraction of non-vicinal secondary alkanediols from leaf waxes of *Papaver alpinum* (Alpine poppy), *P. dubium* (Long-headed poppy), *P. nudicaule* (Iceland poppy), *P. orientale* (Oriental poppy), *P. rhoeas* (Common poppy) and *P. somniferum* (Opium poppy), six hitherto unidentified compounds could be separated by GC. Comparison of their retention data indicated that at least four of them formed a homologous series with chain length differences of two methylene units.

In the respective mass spectra the ions with $m/z = 73$ and 75 showed the presence of at least one OTMSi group [3] and the fragment $m/z = 147$ indicated a diol structure [4]. The alkanediol nature of the compounds was further supported by four α-fragments belonging to two types of ions $[C_nH_{2n}OTMSi]^+$ and $[C_nH_{2n-1}(OTMSi)_2]^+$ [5]. Pairs of these fragments served unambiguously to assign the hydroxyl positions and the overall chain lengths [2]. All six compounds showed the smallest fragment of the first type of ion ($n = 1$; $m/z = 103$), indicative of primary hydroxyl functions [3]. Abundance of this ion is relatively poor as the charge has to be stabilized on a primary carbon. The second hydroxyl group was in all cases located on non-vicinal, secondary carbons. Within pairs of correlated α-fragments the higher mass ion showed lower abundance due to the reduced detector sensitivity in higher mass ranges. In some cases a secondary fragment could be detected that was probably generated from $[C_nH_{2n-1}(OTMSi)_2]^+$ by loss of one HOTMSi ($\Delta m/z = 90$) [2]. The presence of only four α-fragments in each GC peak indicated that all six homo-

logues represented neat isomers, i.e. docosane - 1,3 - diol, tetracosane - 1,3 - diol, hexacosane - 1,7 - diol, octacosane - 1,9 - diol, nonacosane - 1,10 - diol and triacontane - 1,11 - diol. Thus, the six compounds were identified as alkanediols carrying both a primary and a secondary functional group. Correspondingly, their FTIR spectra combined the characteristics of respective spectra from primary and secondary alkanols. To our knowledge none of these compounds has previously been synthesized or identified.

Relative quantities of the new alkanediols were determined by integration and normalization of the GC–flame ionization detector signals of respective OTMSi derivatives (Table 1). Homologues with even carbon numbers prevailed and hexacosane - 1,7 - diol was the only ubiquitous and usually most abundant representative. Tetracosane - 1,3 - diol was detectable (and predominated) only in the leaf wax of *P. orientale*. The new alkanediols amounted to 1% of the extracted wax mixture from *P. alpinum* leaves. In total wax mixtures of the other species investigated the respective isomers were not detectable and it has to be assumed that their proportions did not exceed 0.5%.

The newly identified alkanediols (with the exception of tetracosane-1,3-diol) form a homologous series characterized by constant nonadecyl moieties and with variable numbers of alkylene units between the functional groups ($-CH_2-$ to $-C_9H_{18}-$). Thus, they are distinguished from comparable homologous series of wax components, e.g. α,β-diols and α,ω-diols [6]. It seems noteworthy that both the new alkanediols and the α,β-diols are detectable only in the free form and consist mainly of even-numbered homologues [6] while the main portion of α,ω-diols, consisting equally of even- and odd-numbered homologues, is found esterified in diesters [7] and estolides [8]. Other alkanediols carrying hydroxyl groups on one terminal and on (or near) the central carbon were identified in Eocene oil shales [9] and Quaternary sapropels [10] and have been traced to cyanobacterial origin.

The predominance of even-numbered compounds in the homologous series suggests that they are generated via the elongation–reduction pathway, i.e. the primary hydroxyl function is derived from a carboxyl group [11]. In contrast, the secondary hydroxyl group is probably introduced by oxidation of a precursor hydrocarbon moiety. Similar mid-chain monooxygenase reactions are involved in the biosynthesis of symmetric [12] and asymmetric secondary alkanols [1] or cutin fatty acids [13, 14]. Consequently, both functional groups are inserted in different, i.e. successive reactions (Scheme 1). The order of both hydroxylations cannot be predicted and either primary or secondary alkanols (or their direct precursors) might serve as precursors en route to the respective alkanediols.

The sequence of both hydroxylations may tentatively be judged from the isomer composition of the homologous series. The biosynthesis of nonacosan-10-ol, i.e. of the predominant component in the investigated *Papaver* waxes, probably involves hydroxylation under additive control of the α- and ω-termini with preference of $(\alpha + 9)$ positions [1]. In the new series of alkanediols the mid-chain hydroxyl group is introduced specifically on $(\omega - 19)$ carbons while the second chain terminus has no directing effect, i.e. hydroxylations do not necessarily occur on $(\alpha + 9)$ positions. Therefore, it seems improbable that the pathways leading to asymmetric secondary alkanols and to the new diols share an intermediate containing the secondary hydroxyl group. On the other hand, the distribution of alkanediol homologues roughly follows the relative proportions of the primary alkanol homologues in respective waxes [1]. Consequently, the primary alkanols might serve as precursors and the primary hydroxyl group should be generated before the secondary hydroxyl function (Scheme 1).

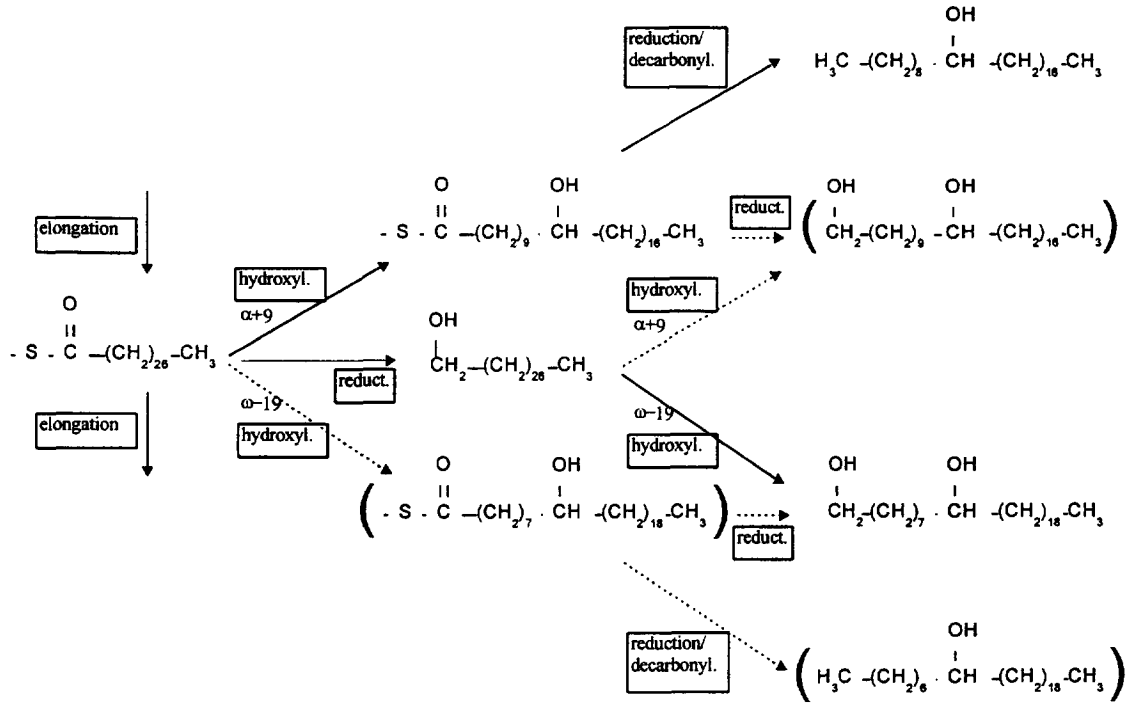
EXPERIMENTAL

Plant wax alkanediols. Plant material was grown from seeds as described earlier [1]. At the state of fruit capsule maturity, 3–5 leaves were cut and immersed ×2 for 30 sec in $CHCl_3$ at room temp. The resulting solns of cuticular waxes were dried, filtered and the solvent removed under red. pres. Substance classes were sepd by TLC [silica gel, mobile phase $CHCl_3$–EtOH (99:1), staining with primuline and UV]. The alkanediol band (R_f 0.01) was removed from the plates and eluted with $CHCl_3$. Compounds containing OH groups were converted (with bis-*N,N*-trimethylsilyltrifluoroacetamide in pyridine for 30 min at 70°) into TMSi derivatives. Qualitative analyses were car-

Table 1. Homologous and isomer composition (%) of alkanediols carrying a primary and a secondary hydroxyl function in *Papaver* leaf waxes

Carbon number	Hydroxyl positions	*P. alpinum*	*P. dubium*	*P. nudicaule*	*P. orientale*	*P. rhoeas*	*P. somniferum*
22	1,3	—	—	—	22	—	—
24	1,3	—	—	—	56	—	—
26	1,7	31	tr*	41	22	tr*	75
28	1,9	54	—	27	—	—	14
29	1,10	—	—	—	—	—	5
30	1,11	15	—	32	—	—	6

*Traces, i.e. less than 0.5% detectable.



Scheme 1. Proposed biosynthetic pathways leading to secondary alkanol and primary/secondary alkanediol isomers in Papaveraceae leaf cuticular waxes. Only the modification of the C_{28} acyl chain is depicted as an example. The absence of possible product isomers (in brackets) makes corresponding pathways improbable (dashed arrows). Sequential ($\alpha + 9$)-hydroxylation of the acyl thioester and reduction/decarbonylation would selectively yield the predominant 10-isomer of heptacosanol. Sequential reduction of the acyl precursor and ($\omega - 19$)-hydroxylation of the intermediate primary alkanol can account for the specific formation of the present diol isomer octacosane-1,9-diol.

ried out by GC-MS and GC-FTIR spectroscopy (30 m OV-1 WCOT, i.d. 320 μm, on-column injection at 50°, over 2 min at 50°, 40° min^{-1} to 200°, 2 min at 200°, 3° min^{-1} to 300°, 30 min at 300° and He carrier gas inlet pressures 8 min at 40 kPa, 2 kPa min^{-1} to 150 kPa, 8 min at 150 kPa), quantitation of individual compounds by GC-FID (as above, but carrier gas H_2).

GC-FTIR ν_{max} cm^{-1} (TMSi ether): 2933, 2863, 1462, 1263, 1104, 845, 759. GC-EIMS [*m/z* (rel. int.)]. Docosane-1,3-diol $[M-15]^+$ 471 (1), 396 (1), 369 (17), 219 (100), 147 (28), 144 (18), 129 (12), 103 (47), 73 (32). Tetracosane-1,3-diol $[M-15]^+$ 499 (1), 424 (1), 397 (14), 219 (100), 147 (29), 144 (19), 129 (14), 103 (48), 73 (67). Hexacosane-1,7-diol $[M-15]^+$ 528 (0.5), 453 (1), 369 (73), 275 (100), 149 (30), 147 (25), 129 (35), 103 (33), 75 (53), 73 (90). Octacosane-1,9-diol $[M-15]^+$ 556 (0.1), 481 (0.2), 369 (47), 303 (100), 149 (22), 147 (16), 129 (17), 103 (15), 75 (35), 73 (62). Nonacosane-1,10-diol $[M]^+$ missing, 369 (100), 317 (62), 149 (26), 147 (21), 129 (25), 103 (22), 75 (57), 73(84). Triacontane-1,11-diol $[M]^+$ missing, 369 (100), 341 (50), 149 (39), 147 (36), 129 (26), 103 (29), 75 (52), 73 (91).

Synthesis of octacosane-1,9-diol. All intermediates were characterized by ^1H (200 MHz) and ^{13}C (50 MHz) NMR spectroscopy. Octane-1,8-diol was reacted with aq. HBr and continuously extracted with hexane over 3 days to give crude 8-bromo-octan-1-ol in 96% yield [15]. The alcohol was protected as its tetrahydropyranyl ether with dihydropyran in presence of triphenylphosphine HBr [16] yielding 2-(8-bromo-octyloxy) tetrahydropyran (95%). Eicosan-1-ol was oxidized to the corresponding aldehyde (84%) with dicyclohexylcarbodiimide in DMSO [17]. Grignard reagent of 2-(8-bromo-octyloxy) tetrahydropyran was reacted with eicosanal to give 1-(tetrahydropyran-2-yloxy) octacosan-9-ol (68%). Deprotection of the tetrahydropyranyl group was effected by using Amberlyst H-15 in a MeOH soln of the tetrahydropyranyl ether [18]: Octacosane-1,9-diol was obtained (79%) as solid: mp 94–95°; GC-FTIR ν_{max} cm^{-1} (TMSi ether): 2934, 2866, 1457, 1261, 1101, 848, 757. GC-EIMS [*m/z* (rel. int.)] $[M-15]^+$ 556 (1), 481 (1), 369 (100), 303 (99), 149 (19), 147 (14), 129 (23), 103 (18), 75 (42), 73 (70). ^1H NMR (200 MHz, $CDCl_3$): δ 0.89 (3H, *t*, $J = 6.4$ Hz, H-28), 1.26 (44H, *br, s*, H-3–H-7, H-11–H-27), 1.59 (6H, *br, s*, H-2, H-8, H-10), 3.55–3.70 (1H, *m*, H-9), 3.64 (2H, *t*, $J = 6.5$ Hz, H-1). ^{13}C NMR (50 MHz, $CDCl_3$): δ (C-1–C-28) 14.1, 22.7, 25.6, 25.7, 29.4, 29.7, 31.9, 32.8, 37.5, 37.6, 63.0, 72.0. Found: C, 78.68; H, 13.85. $C_{28}H_{58}O_2$ required: C, 78.80; H, 13.70%.

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