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LONG-CHAIN POLYPRENYL ACETATES IN *MURRAYA EXOTICA*

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Key Word Index—*Murraya exotica*; Rutaceae; polyprenyl acetates.

Abstract—Polyprenyl acetates with an average number of isoprene residues of eight to 13 were isolated from the leaves of *Murraya exotica*, the content being 0.12% of the dry weight. Spectroscopic analysis revealed that all the polyprenyl acetates were long-chain homologues with the following sequence of isoprene residues: *ω-trans*, two-*trans*, four to nine *cis* and *cis-α*-terminal.

INTRODUCTION

The Rutaceae comprises many genera with important economic and medicinal uses. *Murraya* species have different folk uses especially in India, Australia and South Africa [1, 2]. Roots of *M. exotica* are used as a painkiller, whilst the leaves and bark are used in the treatment of diarrhoea and dysentry [3]. This species is also reported as one of several rutaceous plants used successfully to treat cancer [4].

In previous publications, we have reported the isolation of methoxyflavones [5, 6], coumarins, cycloartenols [7–10] and alkaloids [11, 12]. In continuation of our investigation of the constituents of *M. exotica* collected from the Botanic Island in Aswan (upper Egypt), the author reports the presence of polyprenyl acetates in the leaves (constituting *ca* 0.12% of the dry wt) with the structure **1b**, where $m = 5–10$. The arrangement of the two *trans* (*ω-trans-trans*) and *cis* residues indicates that the isolated polyprenyl acetates are relatively long-chain homologues of betulaprenols [13]. These polyisoprenoids have been used as starting materials for the synthesis of mammalian dolichols (**2**, $m = 12–19$, mainly 14–16) [14–16] which are now recognized as one of the most important materials in the biosynthesis of glycoproteins [17, 18].

In a search for a source of dolichols, many authors [19–22] have investigated the chain-length distribution of polyprenols in several species belonging to the Pinaceae and Ginkgoaceae. These results showed that the polyprenols of the Pinaceae are homologues having an alignment of *ω-trans-trans* residues followed by poly *cis* residues.

RESULTS AND DISCUSSION

UV spectral analysis of the isolated compounds showed no characteristic bands above 210 nm. IR spectral data showed the following absorption bands: 1665 (C=C), 1005 (=CH), 840 (C=C–H) and 1715 cm^{-1} (CO).

FD-mass spectrometry of the isolated compounds showed a fragmentation pattern characteristic of polyisoprenoids [27], *viz.* fragments at *m/z* 135, 202, 271, 339, 407, 543, etc., indicating successive losses of isoprene units from the original compounds. The $[M]^+$ of A–F appeared at *m/z* 604, 672, 740, 808, 876 and 944, corresponding to polyprenyl-8 to -13 acetate of the general formula **1b** ($m = 5–10$). On saponification, the resulting polyprenols gave $[M]^+$ of *m/z* 562, 630, 698, 766, 834 and 902 for A–F, respectively, corresponding to the general formula **1a** ($m = 5–10$).

^{1}H NMR spectral data of **A–F** showed allylic methyl signals at δ1.59, 1.67 and 1.75, assignable to methyl groups of the internal *trans*-isoprene residue and a terminal methyl group *cis-* to the main carbon chain [23], a methyl group of the internal *cis*-isoprene residue and a terminal, methyl group of the α-terminal *cis*-isoprene residue, respectively [23].

^{1}H NMR spectra of the isolated polyprenyl acetates were similar to those of the polyprenols produced by saponification, except that the =CH and $-CH_2OAc$ protons in the α-terminal residue exhibited signals at δ5.35 (*t*, $J = 7.3$ Hz) and 4.55 (*d*, $J = 7.3$ Hz), respectively; these signals were shifted to δ5.44 and 4.08 after saponification of the isolated polyprenyl acetates. ^{1}H NMR spectral data can be used for chain-length determination using integration [22, 24] (Table 1). The relative intensities of signals in the isolated compounds were in good agreement with the theoretical one for structure **1b**. This indicates that the principal polyprenyl acetates (**A–F**) were composed of two internal *trans* residues, two to nine internal *cis* residues and a *cis-α*-terminal residue.

The ^{13}C NMR spectrum is essential for the determination of the internal *cis-* and *trans*-isoprene residue alignment [23]; the α-terminal residue exhibited signals at 32.4

Table 1. Relative intensities of ^{1}H NMR signals of isolated compounds

Compound	No. of isoprene units	$[M]^+$ m/z	Molecular formula	Chemical shift (δ-values) and assignment						
				1.59 Me *trans*, ω (*trans*)	1.67 Me *cis*, ω(*cis*)	1.75 Me α(*cis*)	4.52 4.58 $\underline{CH_2}OH$	5.12 =CH–	5.29 5.33 5.40 $=\underline{CH}CH_2OH$
A	8	604	$C_{42}H_{68}O_2$	3.13*(3)	4.88*(5)	1.03*(1)	1.98*(2)	7.01*(7)	0.97*(1)
B	9	672	$C_{47}H_{76}O_2$	2.94*(3)	6.12*(6)	0.96*(1)	2.02*(2)	7.80*(8)	1.20*(1)
C	10	740	$C_{52}H_{84}O_2$	3.19*(3)	6.79*(7)	0.97*(1)	2.04*(2)	8.73*(9)	1.29*(1)
D	11	808	$C_{57}H_{92}O_2$	3.24*(3)	7.75*(8)	1.07*(1)	1.98*(2)	9.82*(10)	1.27*(1)
E	12	876	$C_{62}H_{100}O_2$	3.17*(3)	8.85*(9)	1.06*(1)	2.07*(2)	10.90*(11)	1.00*(1)
F	13	944	$C_{67}H_{108}O_2$	2.98*(3)	10.11*(10)	0.95*(1)	2.04*(2)	11.82*(12)	1.20*(1)

Theoretical values in parentheses.
Observed and theoretical values for Me protons are the number of methyl groups.

(C-1), 142.4 (C-2), 119.3 (C-3), 61.0 (C-4), 170.6 (Me–CO) and 20.9 (Me–CO). The C-1 methylene carbons exhibited signals around δ32–40 reflecting the linkage of the *cis*- and *trans*-isoprene residues where carbon atoms are designed as follows [24]: the signal at δ39.7 is assigned to C-1 methylene carbons of the *trans*-isoprene residue in the *trans-trans* and *trans* linkage. The signals at δ32.2 are assigned to C-1 methylene carbons of the *cis*-isoprene residue in *cis-cis* and *trans-cis* linkages, respectively. The absence of signals around δ40, which is characteristic for a *cis-trans* linkage, indicates that the *trans*-isoprene units are incorporated in a ω-*trans-trans* linkage. The presence of the ω-*trans* linkage is also confirmed by close inspection of the characteristic C-2 signal of the terminal unit at δ131.1 (the ω-*trans* linkage in model compounds showed a signal around δ131.0–131.3 while the ω-*cis*-linkage showed a signal around δ131.5–131.6) [25]. The relative intensities of the C-1 methylene signals of the isolated compounds were in good agreement with the theoretical values obtained according to the general structure **1b** (Table 2). This indicates that the ω-terminal, two *trans* residues, four to nine *cis* residues and *cis*-α-terminal are aligned in that order.

Reviewing the current literature, it was found that all the polyprenols isolated from the needles or leaves of Gymnosperms investigated so far are long-chain homologues of the betulaprenol-type (**1a**, average $m \geqslant 12$). On the other hand, no such polyprenols have been found in the leaves of Angiosperms in which the dominant polyprenols are relatively short-chain homologues (average $m \leqslant 8$) of the ficaprenol-type in which the internal three *trans*-residues are aligned ω-*trans-trans-trans* [22, 26].

The present study is the first report of polyprenyl acetates of the betulaprenol-type, having 8–13 isoprene units in their skeletons, not only in the Rutaceae but also in Angiosperms.

EXPERIMENTAL

General. UV were recorded in MeOH, IR as KBr pellets. ^{1}H NMR spectra were determined in $CDCl_3$ at 400 MHz using TMS as int. standard. ^{13}C NMR spectra were recorded at 100 MHz ($CDCl_3$, TMS). MS were recorded at 70 eV. Reverse phase HPLC: UV detector, Lichrosorp RP-18. CC: alumina (Merck). TLC: silica gel G (Merck), cyclohexane–EtOAc (9:1).

Isolation and purification. Air-dried powdered leaves of *M. exotica* (5 kg) collected in October from the Botanic Island of Aswan (upper Egypt) were extracted with cyclohexane. After concn under red. pres., 10 g of this extract was chromatographed on an alumina column (4.5 × 75 cm × 1 kg) using cyclohexane–EtOAc (9:1). The oily fr. (R_f 0.51) was chromatographed by HPLC after unsuccessful trials for isolating its components by normal chromatographic methods. Reversed-phase prep. RP-18 column, MeOH–EtOH (3:1) flow rate 3 ml min^{-1}, UV detector at 215 nm.

Table 2. Relative intensities of C-1 methylene carbon signals in the isolated compounds.

Compound	No. of isoprene residues	Chemical shift [δ-values] and assignment			
		32.0	32.20	32.40	39.70
A	8	1.02(1)	3.01(3)	0.91(1)	2.10(2)
B	9	1.07(1)	4.40(4)	1.26(1)	2.10(2)
C	10	1.11(1)	5.03(5)	0.93(1)	1.98(2)
D	11	0.94(1)	5.90(6)	1.05(1)	2.08(2)
E	12	1.20(1)	7.01(7)	0.97(1)	1.95(2)
F	13	0.89(1)	7.89(8)	0.95(1)	2.13(2)

Theoretical values in parentheses.

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