

THE OCCURRENCE OF POLYISOPRENOIDS IN THE LEAVES OF *STERCULIA RUPESTRIS* BENTH.

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نبات الاستركوليا روبسترس بنت ينتمي إلى جنس الاستركوليا وهو أحد أجناس العائلة الاستركوليسية ونظرا للاستخدامات العديدة للنباتات التابعة لهذا الجنس وخصوصا في بعض بلدان أفريقيا ودول شرق آسيا والصين حيث أنها تستخدم كمدرات للبول ومضادات لبعض الفطريات وعلاج بعض الأمراض القاتلية والأزمات الربوية والروماتزم. وقد سبق لنا فصل عدة مركبات فلاونويدية من أوراق هذا النبات وتم أيضا دراسة تأثير المخاط الموجود في أوراقه على سكر الدم في قران التجارب وثبت تأثيره الفعال حيث أنه وجد أنه يخفض سكر الدم بنسبة تصل إلى ٣٠٪ بعد ساعتين.

في هذا البحث تم فصل أربعة مركبات متعددة البرينولات طويلة السلسلة من أوراق النبات في صورة خلات ويتراوح عدد وحدات الأيزوبرين فيها من ٩-١٢ وحدة. وبدراسة تركيب هذه النوعية من المركبات كيميائيا وطيفيا وجد أن ترتيب وحدات الأيزوبرين فيها كما يلي: اوميجا-ترانس، ٢- ترانس، ٥-٨-سيس و سيس-الفا-طرفية- وتنتمي هذه النوعية من المركبات إلى البيتولابرينولات. وهذه الدراسة تمثل أول فصل لهذه النوعية من المركبات بهذا العدد من وحدات الأيزوبرين من نبات الاستركوليا روبسترس. وترجع أهمية هذه المركبات إلى أنها تستخدم كمواد أولية لصناعة الدليكولات والتي تعتبر ذات أهمية كبيرة في التخليق الحيوي للجليكوبروتين.

Polyisoprenyl acetates with an average number of isoprene residues of 9-12 were isolated from the leaves of Sterculia rupestris Benth. (Sterculiaceae). Spectroscopic analysis revealed that all the polyisoprenyl acetates were long-chain homologues with the following sequence of isoprene residues: ω -trans, two-trans, five to eight cis and cis- α -terminal.

INTRODUCTION

In the course of investigation on the constituents of *Sterculia rupestris* Benth., we previously reported the presence of flavonoid glycosides in the leaves.¹ Further investigation of the leaves of the plant resulted in the isolation of a series of four polyisoprenoids.² The arrangement of the two trans (ω -trans-trans) and cis residues indicates that the polyisoprenyl acetates are long-chain homologues of betulaprenols previously reported in gymnosperms.^{2,11} These polyisoprenoids have been used as starting materials for the synthesis of mammalian dolichols (2, m= 12-19, mainly 14-16)^{3,5} which are recognized as one of the most important materials in the biosynthesis of

glycoproteins.^{6,7,16,17}

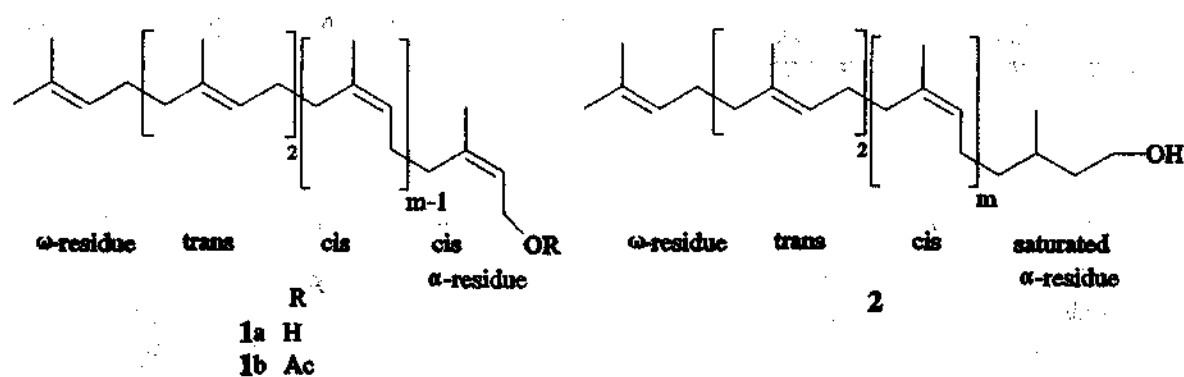
In a research for a source of dolichols; many authors have investigated the chain-length distribution of polyprenols in several species belonging to different families.^{8-11,16,17}

This work is concerned with the identification and structural determination of four polyisoprenyl acetates in the leaves with the structure 1b; where m= 6-9.

EXPERIMENTAL

General

IR spectra are measured in KBr pellets (Berkin-Elmer, 457). ¹HNMR spectra were determined in CDCl₃ at 90 MHz (FX 90Q (Jeol) from Brucker) using TMS as int. standard.



^{13}C NMR spectra were recorded at 100 MHz (CDCl_3 , TMS); GX 400 (Jeol) from Bruker. FD-MS were recorded at 70 eV; Finnigan 4000. Reversed phase HPLC: UV detector, Lichrosorp RP-18. CC: alumina (Merck). TLC: silica gel G (Merck), cyclohexane-EtOAc (9:1).

Plant material

Air-dried powdered leaves of *S. rupestris* (3 kg) were collected from the plants cultivated in the Experimental Station, Faculty of Agriculture, Assiut University, Assiut. The plant was identified by Prof. A. Fayed, Dept. of Plant Taxonomy, Faculty of Science, Assiut University.

Isolation and purification

The dried leaves were extracted with cyclohexane. After concentration under reduced pressure, 70 g of the residue was chromatographed on an alumina column (4x75 cm x 750 g) using cyclohexane-EtOAc (9:1) as eluent. An oily fr. 370 mg, R_f 0.50 (sprayed with 10% H_2SO_4 giving violet colour) 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. The obtained compounds are oily in nature, yellowish in colour with faint odour, oily taste and having the same R_f value of the mixture.

RESULTS AND DISCUSSION

IR spectral data showed the following

absorption bands: 1715 (CO), 1665 (C=C), 1005 (=CH) and 840 cm^{-1} (C=C-H).

FD-mass spectroscopy of isolated compounds showed a fragmentation pattern characteristic for polyisoprenoids,¹² viz. fragment at m/z 135, 202, 271, 339, 407, 543, etc., indicating successive losses of isoprene units from the original compounds. The $[\text{M}]^+$ of 1-4 appeared at m/z 672, 740, 808 and 876, corresponding to polyprenyl-9 to -12 acetates of the general formula 1b ($m = 6-9$). On saponification of the isolated compounds with alcoholic potassium hydroxide,¹⁷ the resulting polyisoprenols gave $[\text{M}]^+$ of m/z 630, 698, 766 and 834 for 1-4 respectively, corresponding to the general formula 1a ($m = 6-9$).

^1H NMR spectral data of 1-4 showed allylic methyl signals at δ 1.59, 1.67 and 1.75, assignable to methyl groups of the internal trans-isoprene carbon chain,¹³ a methyl group of the internal cis-isoprene residue and a terminal methyl group of the α -terminal cis-isoprene residue respectively.¹³

^1H NMR spectra of the isolated compounds were similar to those of the polyisoprenols produced by saponification except that the =CH and - CH_2OAc protons in the α -terminal residue exhibited signals at δ 5.35 (t, $J = 7.3$ Hz) and 4.55 (d, $J = 7.3$ Hz) respectively; these signals were shifted to δ 5.44 and 4.08 after saponification of the isolated polyisoprenyl acetates. It is evident that ^1H NMR spectral data has been used for chain-length determination using integration^{11,14} (Table 1). The relative intensities of signals in the isolated compounds

Table 1: Relative intensities of ¹HNMR 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)	4.22 4.58 CH ₂ OH	5.12 =CH-	5.29 5.33 5.40 =CHCH ₂ OH
1	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)
2	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)
3	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)
4	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)

Theoretical values in parentheses.

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

were in good agreement with the theoretical one for structure 1b. This indicates that the principle polyisoprenyl acetates 1-4 were composed of 2 identical trans residues, two to nine internal cis residues and a cis- α -terminal residue.

^{13}C NMR spectral analysis is essential for the determination of the internal cis- and trans-isoprene residue alignment;¹³ the α -terminal residue exhibited signals at 32.4 (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 δ 32-40 reflecting the linkage of the cis- and trans-isoprene residues where carbon atoms are designed as follows:¹⁴ the signal at δ 39.7 is assigned to C-1 methylene carbons of the trans-isoprene residue in the trans-trans and trans linkage. The signals at δ 32.2 are assigned to C-1 methylene carbons of the cis-isoprene residue in cis-cis and trans-cis linkage, respectively. The absence of signals around δ 40, which is characteristic for a cis-trans linkage, indicates that the trans-isoprene units are incorporated in an 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 δ 131.1 (the ω -trans linkage in model compounds showed a signal around δ 131.0-131.3 while the ω -cis-linkage showed a signal around δ 131.5-131.6).¹⁵ 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, five to eight cis residues and cis- α -terminal are aligned in that order.

The present study is the first report of polyisoprenyl acetates of the betulaprenol-type, having 9-12 isoprene units in their skeletons in *Sterculia rupestris*.

Acknowledgement

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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
1	9	1.07(1)	4.40(4)	1.26(1)	2.10(2)
2	10	1.11(1)	5.03(5)	0.93(1)	1.98(2)
3	11	0.94(1)	5.90(6)	1.05(1)	2.08(2)
4	12	1.20(1)	7.01(7)	0.97(1)	1.95(2)

Theoretical values in parentheses.

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