Phytochemistry, Vol. 34, No. 5, pp. 1335–1339, 1993
Printed in Great Britain.

0031-9422/93 $6.00 + 0.00

OCCURRENCE OF C_{40}–C_{130} POLYISOPRENOID ALCOHOLS IN LOWER PLANTS

TOMÁŠ ŘEZANKA and VALERY M. DEMBITSKY*†

Institute of Microbiology, Vídeňská 1083, 14220 Prague, Czech Republic; *Institute of Ecology of the Volga River Basin, Togliatti 445003, Russia

(*Received in revised form* 27 *April* 1993)

Key Word Index—polyprenols; mosses; liverworts; lichens; algae.

Abstract—Lower plants, i.e. mosses, liverworts, algae and lichens, were found to contain several classes of polyisoprenoid alcohols in the range C_{40}–C_{130}. These were isolated by means of reverse phase-HPLC and identified by EI-mass spectrometry, field desorption-mass spectrometry, IR spectroscopy and ^1H and ^{13}C NMR spectroscopy.

Mosses contain predominantly betulaprenols (polyprenols with two *trans* and the rest *cis* isoprene residues) with average chain length C_{60}. The liverwort examined had two maxima of chain lengths, the first at C_{70} and the second at C_{115}. It contained predominantly ficaprenols (three *trans* and the rest *cis* isoprenes) with minor amounts (up to 5%) of some betulaprenols. Algae have a similar composition to mosses, only the average chain length is moved to higher values ($\sim C_{70}$). Lichens were different in average chain length and in composition of long chain alcohols. They contain ficaprenols with two maxima, the first at C_{80} and the second at $C_{105-110}$ and dolichols (two *trans*, many *cis* and one saturated isoprene residue).

INTRODUCTION

Polyisoprenoid alcohols appear to be universally present in bacteria, plants and animals. The bacterial polyprenols show little diversity of chain length, i.e. predominantly C_{55} [1]. The plant kingdom is the most explored. The plant polyprenols show large differences in chain lengths and the amounts present in different tissues. They range from mixtures of C_{30-40} prenols (wood of birch) [2] through longer chain prenols (C_{50-60}, angiosperm and C_{70-80}, gymnosperm) to prenols of C_{130} and more (e.g. *Araucaria* [3]). Many plants contain di-*trans*-poly-*cis*-prenols (betulaprenols) while others contain tri-*trans*-poly-*cis*-prenols (ficaprenols) [4]. In the case of animal prenols [5] (dolichols), the carbon chains are limited to chain lengths from C_{85} to C_{105}. In some cases the spectrum is broader, but it is never as diverse as shown for plants. All the dolichols are di-*trans*-poly-*cis*-prenols with an α-isoprene saturated unit. The presence of dolichols in plants was reported in 1984 for the seeds of monocotyledons [6]. The presence of a mixture of betulaprenols and ficaprenols in one organism has been reported only once [7]. In the over 30-year history of the studies on the role of polyprenol derivatives as intermediates in the formation of glycoconjugates, the knowledge and the use of plant polyprenols has been of great importance. This paper presents data on the dolichols and polyprenols in lower organisms, their large diversity in chain length and type of prenols. It also describes the use of modern chemico-physical methods (RP-HPLC, IR, ^1H NMR, ^{13}C NMR, EI-MS and FD-MS) for the determination of their structures.

RESULTS AND DISCUSSION

The results obtained for the quantitative estimation of the long chain prenols in the mosses, liverworts, algae and lichens used in this study are given in Table 1. The plants examined contained varying amounts of prenols. The highest amounts (0.01% dry wt) were found in algae, with *Chara fragilis* containing 0.018% prenols on a dry weight basis. The relative proportions of prenols varied widely, as seen in Table 1. Prenols were obtained from the nonsaponifiable fraction obtained on alkaline hydrolysis of the lipid extract of each organism [6]. They were purified by TLC and then separated into individual molecular species by RP-HPLC (Table 1).

Field desorption-mass spectrometry of the prenol mixtures gave the same distribution of molecular ions as the chain length distribution determined by RP-HPLC (Table 1). The mass numbers (m/z) were in keeping with theoretical values for the given spectrum of prenols. Literature data [8] are available concerning the comparison of quantification of dolichols by RP-HPLC and FD-MS. Our use of the two methods in conjunction with each other gave similar results. With increasing M_r of individual prenols RP-HPLC exhibited increasing discrimination of the content of individual prenols, probably

†Present address: Department of Organic Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel.

Table 1. Composition of alcohols

Organisms	Content of prenologues																		
	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
M_r^*	562	630	698	766	834	902	970	1038	1106	1174	1242	1310	1378	1446	1514	1582	1650	1718	1786
Mosses																			
D.s.	0.0	1.0	5.9	12.6	29.6	25.9	14.7	6.7	3.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T.t.	0.9	1.8	17.1	31.3	22.7	13.6	7.2	3.6	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P.c.	1.0	5.1	16.3	39.8	18.5	10.2	6.1	2.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A.v.	0.0	0.7	6.6	23.4	37.2	19.7	8.8	2.9	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P.sp.	0.9	2.6	10.4	54.8	18.3	5.2	4.3	2.6	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M.sp.	0.8	3.2	11.2	19.2	33.6	18.4	10.4	2.4	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B.sp.	0.0	0.7	11.6	19.2	23.4	20.5	13.7	6.8	3.4	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A.a.	0.0	0.6	10.2	14.8	26.1	23.9	11.9	6.8	3.4	1.7	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Liverwort																			
M.p.	0.0	0.0	0.0	0.7	3.9	13.7	24.6	7.2	2.6	0.7	0.7	2.0	3.9	5.2	7.8	12.6	9.7	4.1	0.6
Algae																			
C.f.	0.0	0.0	0.0	0.8	4.6	13.7	38.9	20.5	11.5	6.9	2.3	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A.sp.	0.0	0.0	0.6	3.1	7.4	15.3	23.9	19.6	14.1	8.0	4.9	2.5	0.6	0.0	0.0	0.0	0.0	0.0	0.0
N.o.	0.0	0.6	2.5	5.0	10.0	17.5	26.3	18.1	11.9	5.6	1.9	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lichens																			
A.c.	0.0	0.0	0.0	0.5	2.5	5.4	9.4	12.8	22.2	13.3	5.4	2.5	3.9	8.9	5.9	3.8	2.0	1.0	0.5
A.t.	0.0	0.0	0.4	1.3	3.9	9.9	13.8	17.7	22.8	7.3	2.6	0.9	1.3	3.9	6.9	3.4	2.2	1.3	0.4
E.m.	0.0	0.0	0.8	1.6	3.3	3.7	6.1	10.2	19.2	13.5	8.6	3.3	3.7	9.5	7.8	4.7	2.4	1.2	0.4
H.p.	0.0	0.0	0.0	0.0	0.6	1.7	5.0	12.6	19.5	10.7	2.8	1.7	4.4	8.0	12.6	10.6	5.8	3.5	0.5
C.sp.	0.0	0.0	0.4	1.7	3.4	8.0	11.0	12.2	17.3	10.5	6.8	3.4	1.3	2.5	8.0	6.3	5.1	1.7	0.4

*M_r by FD-MS, see text.

D.s. *Dicranum scoparium*; T.t. *Thuidium tamarisunum*; P.c. *Polytrichum commune*; A.v. *Anomodon veticulosus*; P.sp. *Plagiomnium* sp.; M.sp. *Mnium* sp.; M.p. *Marchantia polymorpha*; B.sp. *Brachythecium* sp.; A.a. *Atrichum angustatum*; C.f. *Chara fragilis*; A.sp. *Acrosiphonia* sp.; N.o. *Nitellopsis obtusa*; A.c. *Anaptychia ciliaria*; A.t. *Aspicilia transbaicalica*; E.m. *Evernia mesomorpha*; H.p. *Hypogomnia physodes*; C.sp. *Cladonia* sp.

owing to the lower absorbance per mol prenol. Our measurements, under conditions optimized for RP-HPLC and especially for FD-MS provided values which differed by not more than ±5% (relative) from each other. Similar results, although without quantitative evaluation, were obtained in a previous study [9].

Interesting conclusions illustrate the dependence of chain lengths on the per cent proportion of prenols in individual plants. Mosses and algae exhibited a single maximum of chain length distribution whereas liverworts and lichens showed two maxima. Earlier studies [3, 4, 10] also reported on two maxima of chain lengths. The explanation of this phenomenon is not yet available.

Semipreparative RP-HPLC gave in each class of organisms a major peak (cf. Experimental) which was further analysed. In the case of lichens and the liverwort the peaks on the RP-HPLC record were split or slightly broadened. Based on literature data [6, 7] and our measurement, it can be assumed that the peaks do not represent defined chemical compounds. Repeated chromatography eventually separated the two components in the C_{80} peak. The structure of the leading fraction was determined by EI-MS, FD-MS and ^{1}H NMR. Individual hydrogen signals in the ^{1}H NMR spectra corresponded to the values measured in standards, in particular in the case of dolichol with signals at δ3.55 (*t*) belonging to hydrogens on a carbon atom with a primary hydroxyl. The second fraction (of longer R_t) contained a substance with a fully unsaturated chain, as confirmed again by ^{1}H NMR. The presence of both dolichols and prenols in a single plant has been repeatedly described [5, 6, 10, 11] mostly in monocotyledonous plants. RP-HPLC of an extract from liverwort revealed a partial separation of individual peaks. The separation was much smaller than mentioned above. This formed the basis of our assumption that individual components of the mixture have different numbers of *cis* and *trans* double bonds (e.g. a mixture of a ficaprenol and a betulaprenol). After six cycles we succeeded in obtaining two enriched fractions. The fraction with shorter R_t had a signal ratio of Me-*trans* (δ1.60) to Me-*cis* (δ1.67) of 2.15:11.85 whereas in the fraction with longer R_t the analogous ratio was 2.93:11.07. These values appear to indicate that the mixture contained a betulaprenol and a ficaprenol. The distribution of prenols with different numbers of *cis* and *trans* isoprene units among plants is extremely broad. Betulaprenols are found predominantly in gymnosperms while ficaprenols are present in angiosperms [4, 12], but only in a single case, in *Magnolia* leaves, are the two types found side by side [7]. We found their simultaneous occurrence also in the relatively uncommon lower plant, i.e. the liverwort.

Polyprenols isolated from all four plant classes, i.e. C_{60}-betulaprenol (mosses), C_{70}-ficaprenol (algae), C_{80}-ficaprenol (lichens) and C_{80}-dolichol (lichens) exhibited IR absorption bands characteristic for *cis* and *trans* isoprenoid units, for a free primary hydroxyl group and for methyl groups.

Much more information about the structure was provided by NMR. The experimental part gives two ^{1}H NMR spectra belonging to a prenol and a dolichol. Ficaprenol and betulaprenol differed only in the intensity of signals at δ1.60 or 1.67, i.e. signals belonging to *cis*- or *trans*-isoprene units. These values were fully in agreement with earlier ones [9]. The spectrum also exhibited a signal at δ1.62 (ω-terminal *trans* unit) and a small signal at δ1.74 which denotes methyl protons in the α-terminal and *cis*-isoprene unit. Other prenols exhibited the same signals.

The dolichol spectrum exhibited in addition mostly signals belonging to a saturated unit (δ0.85 and 0.92 for the methyl group on the α-unit, multiplet at δ1.33 typical again of a saturated α-unit and a triplet at δ3.55 attesting to the presence of a CH_2OH group). The relative intensities (the ratio of signals area at δ1.60 and 1.67) were in good agreement with calculated values for ficaprenol, betulaprenol and dolichol. Based on these values, we could determine the structures in all four plant classes including mixed samples (see above). Assignation of internal *cis*- and *trans*-isoprene units could also be performed by ^{13}C NMR. Our samples exhibited the same ^{13}C NMR signals as the data published previously [4, 9, 10, 13]. The numbering of atoms on a prenol molecule is shown in Fig. 1 and the Experimental part gives corresponding values. The ^{13}C NMR signals exhibited chemical shifts which agreed with literature data [9, 10, 13]. Methylene carbon atoms gave three signals in the range of δ30–40 which corresponded to the *cis–trans* linkage of isoprene units. The signal at δ39.7 belonged to the methylene carbon atom of a *trans* isoprene unit in a *trans–trans* and ω-*trans* linkage. Signals at δ32.0 and 32.0 were again due to methylene carbon atoms, this time belonging to *cis*-isoprene units in *cis–cis* and *trans–cis* linkage. The absence of a signal around δ40.0 characteristic for *cis–trans* linkage indicated that *trans*-isoprene units were incorporated in a ω-*trans–trans*-linkage [9]. The presence of the ω-*trans*-linkage was also confirmed by the characteristic carbon signal (δ131.4) corresponding to the ω-terminal unit. All these values (for assignation of individual atoms see Fig. 1 and Experimental) were in very good agreement with published data. Based on these values, the prenols found in our work have been

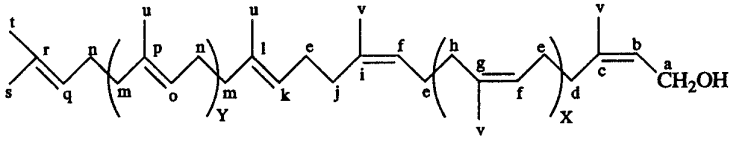

Fig. 1. The structure with numbering of prenols (X=7, Y=1 betulaprenol C_{60}; X=8, Y=2 ficaprenol C_{70}).

assigned structures of ficaprenols, betulaprenols and dolichols (cf. Experimental).

All prenols exhibited a molecular ion (although often very weak). The difference between prenols and dolichols was best demonstrated by the ratios of intensities of the $[M-H_2O]^+$ ions [14], prenols having a much more intensive $[M-18]^+$ ion. As mentioned above, the most intensive ion in the region of the molecular ion was at $[M-18]^+$, which corresponds to the loss of water from a primary alcoholic group. Further splitting occurred on the ω-terminal end, whereby isoprene residues were cleaved off (cf. Experimental). All values for ficaprenols, betulaprenols and dolichols were in good accord with the literature [9, 15].

Numerous studies have been concerned with the occurrence of prenols in higher plants, especially conifers, and mono- and dicotyledons, while studies on lower plants are almost nonexistent. An exception is the study [16] which describes the occurrence of unidentified prenols with probable chain lengths C_{65}–C_{70} in the green alga *Chlorella pyrenoidosa*. No prenols were identified in other algae, liverworts, horsetails and ferns [16]. Thanks to modern separation methods, we identified prenols in all of the plants under study. Three basic tenets can be derived from our results: the presence of prenols in four classes of lower plants out of which mosses and algae exhibit a single chain length maximum while liverworts and lichens have two maxima. Furthermore, lichens contain both dolichols and ficaprenols; this had previously been found only in higher plants, particularly monocotyledons. By contrast, the liverwort contained both betulaprenols and ficaprenols, such simultaneous occurrence having been found only in the dicotyledonous plant *Magnolia*. Although the study was performed on a few species only, the results confirmed the ubiquity of prenols and should thus stimulate further analytical studies, e.g. in the field of chemotaxonomy.

EXPERIMENTAL

Isolation. The plant material was collected from 15 to 25 August 1992 in the Volga river basin near to the town Togliatti. Only parts of lower plants from last year were analysed. The lipid extract from each plant was subjected to alkaline hydrolysis [6] and the nonsaponifiable lipids isolated by means of hexane–Et_2O.

The unsaponifiable fr. was subjected to prep. TLC (Kieselgel plate with EtOAc–C_6H_6, 1:19). The edge of the plate was developed with I_2 and the band corresponding to free long chain alcohols (R_f 0.56) (i.e. betulaprenols, ficaprenols and dolichols) was scraped off, eluted with 10 ml *i*-PrOH–hexane (1:1) and the eluate after evapn to 1/10 vol. was subjected to semiprep. RP-HPLC. Standards (C_{90} dolichol and C_{60} ficaprenol) were obtained from Sigma.

RP-HPLC. A Semiprep. column (250 mm × 8 mm i.d.) packed with SGX C18 with 7 μm particles was used (Tessek, Prague, Czech Republic). After injection of 100 μl soln of total alcohols (50 mg ml^{-1}) a gradient of isopropanol–H_2O–hexane (convex gradient from 41:4:5 to 3:0:7 for 40 min) was applied. The effluent was monitered at 210 nm and the peaks collected manually. The alcohol (C_{80}) had R_t 24.5 min. The major peaks from RP-HPLC of mosses, liverworts, lichens and algae were subjected to further analysis, i.e. IR, NMR and EI-MS.

Separation of peak 16 *(lichens) into two components—ficaprenol and dolichol.* The total fr. (~800 μg) was subjected to RP-HPLC (as above). The partially split peak was divided into 2 frs, which were recycled repeatedly. After 4 cycles 2 frs were obtained. The first fr. contained ficaprenol and the second dolichol; purity *ca* ~90–95% (by FD-MS; $[M]^+$ *m/z* 1106 and $[M]^+$ *m/z* 1108). The structures were confirmed by ^{1}H NMR and ^{13}C NMR.

Separation of peak 14 *(liverwort) into two compounds—ficaprenol and betulaprenol* (3T+11C, *respectively*, 2T+12C). Carried out as just described. Two frs were obtained, but only enrichment was obtained. The structures were confirmed by EI-MS, FD-MS and ^{1}H NMR.

IR. Sample (50 μg) in CCl_4, ν_{max} cm^{-1}: 3400 (OH), 2970, 2860, 1448 (Me), 2930, 1376 (CH_2), 3020, 1660, 835 (C=C), 1060 (C–O).

Mass spectrometry. Field desorption mode with a Finnigan MAT 90 (Finnigan, F.R.G.). An emitter current of 15 mA was used to desorb the sample. The sample was dissolved in hexane and then loaded on to the emitter. EI-MS *m/z* (rel. int.) C_{60} betulaprenol: 834 (2.2; $[M]^+$), 816 ($M-H_2O$; 6.9), 815 ($M-H-H_2O$; 0.4), 747 ($M-H-H_2O-i_1$; 1.3), 679 ($M-H-H_2O-i_2$; 1.4), 611 ($M-H-H_2O-i_3$; 1.6), 543 ($M-H-H_2O-i_4$; 2.7), 475 ($M-H-H_2O-i_5$; 6.9), 407 ($M-H-H_2O-i_6$; 1.6), 339 ($M-H-H_2O-i_7$; 12.6), 271 ($M-H-H_2O-i_8$; 23.1), 203 ($M-H-H_2O-i_9$; 42.2), 135 ($M-H-H_2O-i_{10}$; 65.2), 121 (88.5), 95 (93.0), 81 (96.5), 69 (100), 55 (42.6), 41 (59.7); C_{70} ficaprenol: 970 (1.9; $[M]^+$), 952 ($M-H_2O$; 5.4), 951 ($M-H-H_2O$; 0.5), 883 ($M-H-H_2O-i_1$; 1.5), 815 ($M-H-H_2O-i_2$; 1.6), 747 ($M-H-H_2O-i_3$; 1.8), 679 ($M-H-H_2O-i_4$; 2.1), 611 ($M-H-H_2O-i_5$; 2.6), 543 ($M-H-H_2O-i_6$; 2.8), 475 ($M-H-H_2O-i_7$; 3.2), 407 ($M-H-H_2O-i_8$; 3.6), 339 ($M-H-H_2O-i_9$; 6.6), 271 ($M-H-H_2O-i_{10}$; 13.1), 203 ($M-H-H_2O-i_{11}$; 22.3), 135 ($M-H-H_2O-i_{12}$; 67.8), 121 (80.7), 95 (97.3), 81 (99.1), 69 (100), 55 (52.7), 41 (67.2); C_{80} ficaprenol: 1106 (0.9; M^+), 1088 ($M-H_2O$; 3.9), 1087 ($M-H-H_2O$; 0.2), 1019 ($M-H-H_2O-i_1$; 0.6), 951 ($M-H-H_2O-i_2$; 0.7), 883 ($M-H-H_2O-i_3$; 0.9), 815 ($M-H-H_2O-i_4$; 1.1), 747 ($M-H-H_2O-i_5$; 1.5), 679 ($M-H-H_2O-i_6$; 1.7), 611 ($M-H-H_2O-i_7$; 2.0), 543 ($M-H-H_2O-i_8$; 2.1), 475 ($M-H-H_2O-i_9$; 2.5), 407 ($M-H-H_2O-i_{10}$; 3.4), 339 ($M-H-H_2O-i_{11}$; 8.9), 271 ($M-H-H_2O-i_{12}$; 11.3), 203 ($M-H-H_2O-i_{13}$; 29.0), 135 ($M-H-H_2O-i_{14}$; 51.7), 121 (66.7), 95 (92.6), 81 (95.2), 69 (100), 55 (65.7), 41 (70.6); C_{80} dolichol: 1108 (2.9; $[M]^+$), 1090 ($M-H_2O$; 1.6), 1089 ($M-H-H_2O$; 0.7), 1017 ($M-H-H_2O-i_1$; 0.5), 949 ($M-H-H_2O-i_2$; 0.4), 881 ($M-H-H_2O-i_3$; 0.6), 813 ($M-H-H_2O-i_4$; 1.0), 745 ($M-H-H_2O-i_5$; 1.3), 677 ($M-H-H_2O-i_6$; 1.4), 609 ($M-H-H_2O-i_7$; 1.7), 541 ($M-H-H_2O-i_8$; 2.0), 473 ($M-H-H_2O-i_9$; 2.4), 405 ($M-H-H_2O-i_{10}$; 3.2), 337 ($M-H-H_2O-i_{11}$; 8.7), 269 ($M-H-H_2O-i_{12}$; 10.7), 201 ($M-H-H_2O-i_{13}$; 32.4), 133 ($M-H-H_2O-i_{14}$; 64.8), 121

(72.4), 95 (96.1), 81 (91.3), 69 (100), 55 (58.2), 41 (61.0); i = isoprene unit: [$-CH_2=C(Me)=CH-CH_2-$].

^1H *and* ^{13}C *NMR spectra.* Measured on a Brucker AM-300 instrument ($CDCl_3$, ppm, TMS) at 300 or 75 MHz. Betulaprenol: δ1.60 (*s*, 6H, Me *trans*), 1.62 (6H, Me ω-*trans*), 1.67 (24H, Me *cis*), 1.74 (3H, Me α); 2.02 + 2.05 (*ss*, 22H, CH_2–C(Me)=), 4.09 (*d*, 2H, CH_2OH), 5.12 (*q*, 11H, =CH–), 5.43 (1H, =CH–CH_2OH); ficaprenol: 1.60 (*s*, 9H, Me-*trans*), 1.62 (6H, Me ω-trans), 1.67 (27H, Me-*cis*), 1.74 (3H, Me α), 2.02 + 2.05 (*ss*, 26H, CH_2–C(Me)=), 4.09 (*d*, 2H, CH_2OH), 5.12 (*q*, 13H, =CH–), 5.43 (1H, =CH–CH_2OH); dolichol: 0.85 + 0.92 (*d*, 3H, CH_2–CH(Me)–CH_2; e.g. α Me), 1.33 (*m*, 5H, CH_2–CH(Me)–CH_2), 1.60 (*s*, 36H, Me-*trans*), 1.62 (6H, Me ω-*trans*), 1.67 (6H, Me-*cis*), 2.02 + 2.05 (*ss*, 28H, CH_2–C(Me)=), 3.55 (*t*, 2H, sat-CH_2OH), 5.12 (*q*, 15H, =CH–).

^{13}C NMR of prenol (for assignment of carbon, see Fig. 1). a—59.0 (*t*); b—124.7 (*d*); c—139.5 (*s*); d—29.6 (*t*); e—26.5 (*t*); f—125.0 (*d*); g—135.7 (*s*); h—32.0 (*t*); i—134.8 (*s*); j—32.0 (*t*); k—124.2 (*d*); l—135.9 (*s*); m—39.7 (*t*); n—26.8 (*t*); o—124.4 (*d*); p—131.0 (*s*); q—124.5 (*d*); r—131.4 (*s*); s—25.7 (*q*); t—17.6 (*q*); u—16.0 (*q*); v—23.4 (*q*).

REFERENCES

1. Gough, D. P., Kirby, A. L., Richards, J. B. and Hemming, F. W. (1970) *Biochem. J.* **118**, 167.
2. Lindgren, B. O. (1965) *Acta Chem. Scand.* **19**, 1317.
3. Swiezewska, E. and Chojnacki, T. (1988) *Acta Biochim. Polon.* **35**, 131.
4. Ibata, K., Kageyu, A., Takigawa, T., Okada, M., Nishida, T., Mizuno, M. and Tanaka, Y. (1984) *Phytochemistry* **23**, 2517.
5. Hemming, F. W. (1974) in *Biochemistry of Lipids* (Goodwin, T. W., ed.), MTP Int. Rev. Sci., Biochemistry Series One, Vol. 4, pp. 39–97. Butterworths, London.
6. Ravi, K., Rip, J. W. and Carroll, K. K. (1984) *Lipids* **19**, 401.
7. Sasak, W., Mankowski, T. and Chojnacki, T. (1977) *Chem. Phys. Lipids* **18**, 199.
8. Steen, L., Dessel, G. V., Wolf, M. D., Lagrou, A., Hilderson, H. J., Keukeleire, D. D. and Pinkse, F. A. (1984) *Biochim. Biophys. Acta* **796**, 294.
9. Ibata, K., Mizuno, M., Takigawa, T. and Tanaka, Y. (1983) *Biochem. J.* **213**, 305.
10. Jankowski, W. J. and Chojnacki, T. (1991) *Acta Biochim. Pol.* **38**, 265.
11. Ravi, K., Rip, J. W. and Carroll, K. K. (1983) *Biochem. J.* **213**, 513.
12. Stone, K. J., Wellburn, A. R., Hemming, F. W. and Pennock, J. F. (1967) *Biochem. J.* **102**, 325.
13. Monaco, P., Previtera, L. and Belardini, M. (1983) *J. Nat. Prod.* **46**, 174.
14. Richards, J. B. and Hemming, F. W. (1972) *Biochem. J.* **128**, 1345.
15. Wellburn, A. R., Stevenson, J., Hemming, F. W. and Morton, R. A. (1967) *Biochem. J.* **102**, 313.
16. Wellburn, A. R. and Hemming, F. W. (1966) *Phytochemistry* **5**, 969.