Phytochemistry, Vol. 21, No. 2, pp. 301–303, 1982.
Printed in Great Britain.

0031-9422/82/020301-03$03.00/0

FATTY ACID CHANGES DURING MATURATION OF *MOMORDICA CHARANTIA* AND *TRICHOSANTHES ANGUINA* SEEDS

GOLLAMUDI LAKSHMINARAYANA, T. N. B. KAIMAL, V. V. S. MANI, K. SITA DEVI and T. CHANDRASEKHARA RAO

Regional Research Laboratory (CSIR), Hyderabad 500 009, India

(*Revised received* 22 *June* 1981)

Key Word Index—*Momordica charantia*; *Trichosanthes anguina*; Cucurbitaceae; seed maturation; conjugated fatty acids.

Abstract—Changes in fatty acids were studied during maturation of *Momordica charantia* and *Trichosanthes anguina* seeds, which contain *cis*-9, *trans*-11, *trans*-13-octadecatrienoic acid (α-eleostearic) and *cis*-9, *trans*-11, *cis*-13-octadecatrienoic acid (punicic), respectively. The two seeds matured 30 and 35 days after flowering, respectively. Total lipids as well as α-eleostearic acid accumulated rapidly from 10 to 20 days in *M. charantia*. In *T. anguina* the active period of lipid synthesis was from 15 to 30 days but punicic acid continued to be synthesized until maturity. In both species, the disappearance of linolenic acid and the reduction in concentration of linoleic acid were concomitant with the formation of conjugated fatty acids. The conjugated fatty acids were absent from monoacylglycerols and phospholipids of both species, and also from the diacylglycerols of *M. charantia*, throughout maturation.

INTRODUCTION

Changes in fatty acids, both common and unusual, during seed maturation have received much attention [1, 2]. However, only one seed source among those containing conjugated octadecatrienoic acids (18:3 conj.), *Momordica balsamina*, which contains punicic acid (*cis*-9, *trans*-11, *cis*-13-octadecatrienoic acid) has been studied before [3]. The changes in fatty acid composition during maturation of two other seeds of the Cucurbitaceae containing 18:3 conj. [4], viz. *Momordica charantia* (α-eleostearic acid, *cis*-9, *trans*-11, *trans*-13-octadecatrienoic acid) and *Trichosanthes anguina* (punicic acid), are reported here.

RESULTS AND DISCUSSION

M. charantia

The lipid content remained small until 10 days after flowering; thereafter there was a rapid accumulation (Table 1). From day 10 to 13 the lipid content increased six-fold and by day 20 it had reached almost the maximum level. Until day 10, 16:0 and 18:2 were the major fatty acids and 18:3 conj. was not found. From day 6 to 10, 16:0 and 18:0 decreased and 18:1, 18:2 and 18:3 increased. By day 13 16:0 further decreased, 18:0 increased, 18:2 decreased, 18:3 disappeared and 18:3 conj. became predominant. By day 20 when the lipid content reached a maximum level, 18:0 and 18:3 conj. also attained almost maximum concentrations. The fatty acid composition remained more or less the same after day 20.

The changes in fatty acid composition of the triacylglycerols (TG) broadly reflected those occurring in total lipids. Most of the 18:3 conj. synthesized was incorporated into the TG. In the diacylglycerols (DG) and polar lipids (PL) of seeds from day 20 onwards, 18:0 predominated and the 18:2 content decreased but 18:3 conj. was absent.

T. anguina

The lipid content was not appreciable until 15 days after flowering; rapid accumulation was observed thereafter until day 30 (Table 2). 18:3 Conj. was absent on day 15, present in appreciable amounts on day 25 and continued to be synthesized even after day 30. Before the appearance of 18:3 conj., 18:2, 16:0 and 18:3 were the major acids. By day 25, 18:3 had disappeared, the 18:2 and 16:0 contents had decreased and the 18:0 and 18:1 contents increased. From the day 25 to 35, the amounts of 18:1 and 18:2 decreased and those of 18:3 conj. increased further whereas those of 16:0 and 18:0 remained the same.

The changes in the TG were generally similar to those in the total lipids. The content of 16:0 in the DG decreased continuously and the contents of 18:0 and 18:1 increased until day 25 and decreased thereafter. The 18:2 content also declined during the ripening period. Appreciable proportions of 18:3 conj. were found in the samples of days 30 and 35. The contents of 16:0 and 18:3 in the PL decreased, those of 18:0 and 18:1 increased until day 30 and the changes thereafter were minor. Throughout, 18:2 was predominant and 18:3 conj. was absent.

The disappearance of 18:3 and reduction in 18:2 concentration in the total lipids, TG and DG of *M. charantia* and *T. anguina* were found to be concomitant with the formation of 18:3 conj. This suggests that the latter could possibly originate from

Table 1. Changes in fatty acid composition during maturation of *M. charantia* seeds

Days after flowering	Lipid (% dry wt)	Lipid	Fatty acids (wt%)					
			16:0	18:0	18:1	18:2	18:3	18:3 conj.*
6	4.1	Total	52.0	12.1	4.6	19.8	11.5	0
10	5.1	Total†	29.7	6.6	11.2	31.8	19.1	0
13	30.2	Total	3.8	21.8	14.8	16.1	Tr	43.4
		TG‡	3.3	25.9	20.6	12.8	Tr	37.4
		DG‡	20.6	23.7	19.5	36.2	Tr	0
		PL‡	27.8	10.6	55.6	6.0	Tr	0
20	46.4	Total	1.5	37.9	1.9	3.5	0	55.1
		TG	2.3	48.8	3.8	4.6	0	40.5
		DG	9.6	58.4	8.2	23.7	0	0
		PL	15.8	66.6	5.3	12.3	0	0
24	47.7	Total	1.8	33.5	2.3	4.8	0	57.4
		DG	11.9	61.5	8.1	18.5	0	0
		PL	18.2	59.4	12.6	9.8	0	0
30	47.5	Total	1.7	36.1	1.3	4.1	0	56.7
		TG	2.2	34.3	16.5	4.5	0	42.5
		DG	24.6	46.2	17.6	11.6	0	0
		PL	29.0	38.7	27.6	4.6	0	0

**Cis*-9, *trans*-11, *trans*-13-octadecatrienoic acid.
†Also contained 12:0, 0.7% and 14:0, 0.9%.
‡TG, triacylglycerol; DG, diacylglycerol; PL, polar lipids (mixture of monoacylglycerols and phospholipids).

Table 2. Changes in fatty acid composition during maturation of *T. anguina* seeds

Days after flowering	Lipids (%, dry wt)	Lipid	Fatty acid (wt%)						
			14:0	16:0	18:0	18:1	18:2	18:3	18:3 conj.*
10	1.8	Total	3.4	27.8	2.9	4.2	37.3	24.3	0
		TG†	10.6	16.8	3.5	5.7	38.9	24.5	0
		DG†	2.9	22.5	4.2	10.2	38.4	21.8	0
		PL†	0	32.6	2.7	2.6	38.0	24.1	0
15	3.1	Total	2.8	28.0	2.2	5.5	38.5	23.0	0
		TG	2.2	18.6	10.0	17.5	34.7	17.0	0
		PL	0	29.7	4.4	5.3	36.5	24.0	0
25	17.4	Total	0	7.5	10.3	28.5	28.2	Tr	25.4
		TG	0	7.8	12.0	32.1	30.4	0	17.7
		DG	0	11.7	19.8	39.6	28.9	0	0
		PL	0	25.5	5.6	14.6	45.5	8.9	0
30	31.3	Total	0	6.1	10.1	24.9	25.5	Tr	33.3
		TG	0	7.1	10.8	25.9	29.1	0	27.1
		DG	0	7.2	10.3	34.4	32.8	0	15.4
		PL	0	20.0	11.7	22.5	43.4	2.4	0
35	32.3	Total	0	7.2	10.1	17.5	19.0	Tr	46.3
		TG	0	3.6	11.1	19.2	18.4	0	47.7
		DG	0	5.4	4.9	17.2	22.2	0	50.2
		PL	0	20.7	7.5	21.7	45.3	4.7	0

**Cis*-9, *trans*-11, *cis*-13-octadecatrienoic acid.
†TG, triacylglycerols; DG, diacylglycerols; PL, polar lipids (mixture of monoacylglycerols and phospholipids).

18:2, as hypothesized by earlier workers [5, 6]. The absence of 18:3 conj. in PL, which include monoacylglycerols (MG) and phospholipids, at any stage of seed maturation suggests that the TG and also the DG of *T. anguina* containing 18:3 conj. may perhaps be synthesized by acylation of the MG with 18:3 conj. and other fatty acids, via the MG pathway [7].

EXPERIMENTAL

M. charantia (cv. Long Green Monsoon) and *T. anguina* seeds were purchased from Pocha Seeds, Pune, India. The plants were grown in prepared plots and pollinated flowers were tagged. Seeds were collected at different stages of maturation. Moisture contents were determined by heating at 110°. Seeds were ground with Na_2SO_4 and extracted ×3 with $CHCl_3$–MeOH (2:1). The combined extracts were concd in a rotary evaporator at 35°, dil. with H_2O and re-extracted with hexane. The extracts were washed ×3 with 10% NaCl soln, and dried over Na_2SO_4.

The TG, DG and PL were separated by prep. TLC on Si gel (1 mm) using hexane–Et_2O (7:3). The TG and DG were extracted with H_2O–satd Et_2O and PL with $CHCl_3$–MeOH (1:1). The MG and phospholipids present in the PL fraction, as shown by Si gel TLC using hexane–Et_2O (1:1), were not separately analysed. The lipids were transesterified with NaOMe–MeOH and the resultant Me esters were analysed by FID GC on 5% SE 30–Chromosorb P, 45–60 (1.2 m × 3 mm) and 15% EGSS–X–Gas Chrom Q, 100–120 (1.8 m × 3 mm) columns. The column, injection port and detector were maintained at 190°, 240° and 240°, respectively. N_2 flow rate was 30 ml/min. Since 18:3 conj. is known to undergo alteration on polyester columns [8] it was estimated by GC on SE-30 and the accuracy of the estimation was verified by analysing *M. charantia* oil methyl esters to which known amounts of methyl stearate were added, as well as by a UV method [9]. That alteration products of 18:3 conj. were not eluted with other esters, as was shown by analysing a mixture of 16:0, 18:0, 18:1, 18:2, 18:3 and 20:0 to which different proportions of α-eleostearate were added. Methyl α-eleosterate was isolated from *M. charantia* oil methyl esters by a double development of Si gel with hexane–Et_2O (47:3).

Acknowledgement—This work was supported by Grant No. FG-In-521, U.S. Department of Agriculture, Far Eastern Regional Research Office, American Embassy, New Delhi, India.

REFERENCES

1. Hitchcock, C. and Nichols, B. W. (1971) *Plant Lipid Biochemistry*, pp. 236–241. Academic Press, New York.
2. Appelqvist, L.-Å. (1975) in *Recent Advances in the Chemistry and Biochemistry of Plant Lipids* (Galliard, T. and Mercer, E. I., eds), pp. 247–286. Academic Press, New York.
3. Lotti, G., Izzo, R. and Nicolini, F. (1973) *Riv. Ital. Sostanze Grasse* **50**, 425.
4. Chisholm, M. J. and Hopkins, C. Y. (1964) *Can. J. Chem.* **42**, 560.
5. Gunstone, F. D. (1966) *Chem. Ind.* 1551.
6. Morris, L. J. and Marshall, M. O. (1966) *Chem. Ind.* 1493.
7. Hirayama, O. and Hujii, K. (1965) *Agric. Biol. Chem. Tokyo* **29**, 1.
8. Morris, L. J., Holman, R. T. and Fontell, K. (1960) *J. Lipid Res.* **1**, 412.
9. *Official and Tentative Methods of the AOCS* (1973) 3rd edn, pp. 7–58.