Pergamon 0031-9422(94)00724-1 Phytochemistry, Vol. 38, No. 3, pp. 585-587, 1995

0031-9422/95 $9.50 + 0.00

LIPID COMPOSITION OF MICROSOMES OF *IRIS GERMANICA* RHIZOMES

JEAN-PAUL BONFILS, CLAUDE BONFILS,* CHRISTIAN LARROQUE,* AGNES SURJUS, DIDIER GLEIZE and YVES SAUVAIRE

Laboratoire de Recherche sur les Substances Naturelles Végétales, EA 729, Université Montpellier II, C-P 024, 34095 Montpellier Cedex 5, France; *INSERM Unité 128, CNRS, BP. 5051, 34033 Montpellier, France

(*Received* 6 *June* 1994)

Key Word Index—*Iris germanica*; Iridaceae; lipid composition; microsomes; phytosterols; triterpenoids; iridals.

Abstract—Analysis of the intracellular membranes of *Iris germanica* rhizomes indicated high amounts of iridals when compared to sterols and lower sterol amounts than in other plant microsomes. Microsomal preparations from other plants and animals were also shown to incorporate high amounts of iridals, with a special affinity for free iridals. The localization of free iridals within the cellular membranous fraction and their sterol-like structural role are discussed.

INTRODUCTION

Iridals are triterpenoid compounds discovered in the last decade [1]. Up to now, more than 20 iridals have been isolated from various *Iris* [1–4] and *Belamcanda* species [5]. These substances may exist in two forms, free iridals (FI) and iridal esters (IE) [1].

In an earlier paper [6], we have shown that FI could contribute to the maintainance of membrane integrity when *Iris* rhizome slices were subjected to dehydration. These investigations also indicated that the phospholipid (PL) composition of *I. germanica* rhizomes was in agreement with that observed in other storage organs [6].

In order to get some insight into the relationship between iridals and cell membranes, an analysis of the lipid composition of *Iris* microsomes was carried out. Thus, only sterols and iridals were investigated during this work.

RESULTS AND DISCUSSION

Analysis by HPLC of lipid extracts from microsomes of *I. germanica* rhizomes revealed important amounts of iridals when compared to sterol contents (Table 1). Similar results were indicated from intact roots, rhizomes and leaves of *I. pallida* [7]. The level of sterols (*ca* 30 $\mu g\,mg^{-1}$ protein) in our uninduced microsomal fraction seems quite low compared to that usually encountered. For example, in potato tuber microsomes (another storage organ) Dupéron *et al.* [8] found *ca* 410 $\mu g\,mg^{-1}$ of protein. However, sterol amounts vary depending on plant, organ and ontogenic stages studied [8]. In oat plasma membranes, Norberg and Liljenberg [9] observed total sterol contents of *ca* 80 $\mu g\,mg^{-1}$ protein.

When studied in terms of dry weight, a comparison between sterol and iridal contents is also very informative. Most of the results reported in literature show a sterol content of *ca* 1–3 $\mu mol\,g^{-1}$ dry weight in intact tissue [7, 10–15] and *ca* 0.15–0.20% dry weight in leaf microsomes [16]. Concerning iridals, previous investigations on crude extracts of *I. germanica* rhizomes indicate *ca* 200 $\mu mol\,g^{-1}$ dry weight [6] and *ca* 4% dry weight [17].

Free sterols (FS) are known to be located inside membranes (for a review see ref. [18]) and some papers indicate a ratio FS/total sterol around 0.90 [19]. In our microsomes, this ratio is *ca* 0.45 (Table 1). Dupéron and Dupéron [16] found that *I. germanica* leaf microsomes contained 73% of FS. Many workers have also reported high amounts of steryl esters (SE) and steryl glycosides (SG) within cell membrane fractions [8, 16, 18].

Incubation of *Iris* rhizome slices with $MnCl_2$ induces cytochrome P-450 synthesis [20]. Total protein content must also be enhanced, since the ratios sterol:protein, as well as iridal:protein dropped significantly in induced microsomes (Table 1). Nevertheless, in both cases, iridal levels are more than 10-fold higher than those of sterols.

Lipid extracts obtained from microsomes, as well as from fresh *I. germanica* rhizomes, were resolved by HPLC. The chromatograms were clearly different and indicated that FI were the main type of iridals in the intracellular membranous fraction. The presence of FI in membranes is consistent with previous results obtained after dehydration of *I. germanica* rhizome slices [6].

The extremely high amounts of FI in *Iris* microsomes are neither likely to result in contamination from the supernatant, nor adsorption on the membrane, since the addition of activated charcoal or polyvinylpyrrolidone to

Table 1. Sterol* and iridal levels in *I. germanica* rhizome microsomes prepared with or without three days cytochrome P-450-inducing treatment with $MnCl_2$

	($\mu g\,mg^{-1}$ Protein)			($\mu g\,mg^{-1}$ Protein)		
	Free sterols (FS)	Total sterols (TS)	FS:TS	Free iridals (FI)	Iridal esters (IE)	FI:IE
Untreated microsomes	15.0 ± 2.5	33.0 ± 5.0	0.45 ± 0.01	428 ± 33	19 ± 2	33.8 ± 5.6
Induced microsomes	9.2 ± 1.5	16.3 ± 1.4	0.56 ± 0.05	298 ± 17	10.6 ± 0.6	28.2 ± 0.30

Results are mean ± s.d. of three replicates.

*Sterol-analysed are: cholesterol (traces), campesterol (14.8% ± 1.8), stigmasterol (9.9% ± 1.3) and sitosterol (74.8% ± 3.6). The relative abundance of sterols is calculated on the untreated microsomes.

the microsome suspensions does not remove iridals from these membrane fractions (data not shown).

With cell cultures of *I. pallida* fed with iridals (dissolved in ethanol), rapid uptake and metabolism of these products was observed [7]. Cholesterol could be readily incorporated within microsomal fractions by ultrasonication of a suspension containing microsomes and cholesterol [21]. In order to confirm the presence of FI in membranes, similar incorporation experiments with (FI + IE) mixtures in microsomes were undertaken.

Microsomes from plant as well as from animal tissues, used for incorporation experiments, were shown previously to be free of iridals. The results obtained indicate that 77.3 ± 2.4% (four replicates) of the iridals are present in the microsomal pellets after incorporation procedures. Furthermore, the rate of incorporation is related to the nature of the iridals. FI are readily incorporated within the microsomal fraction since the ratio FI:IE is always higher in this fraction than in the supernatant, when compared with the control (Table 2).

In conclusion, our results show that FI are intracellular membrane constituents in *I. germanica*. Conversely, we find a low sterol level in these membranes, especially for FS (45% of total sterol). As we demonstrated recently [6], some properties of iridals could contribute to the ability of *Iris* species to colonize difficult habitats, like dry lands (*I. germanica*) or floodable lake sides (*I. pseudacorus*). The present results strongly suggest that iridals could have a structural role within *Iris* membranes comparable to that of sterols. The presence of sterol within membranes and their interaction with PL are due in part to the presence of a C-3 hydroxyl group on the sterol molecule. The substitution of this hydroxyl group with a fatty acid molecule reduces the affinity between sterols and PL. It is likely that esterification of FI, which occurs only at the C-3 hydroxyl group [3] has the same effect, since only small amounts of IE are found in *Iris* microsomes. The sterol-like role of iridals in membrane structure needs further confirmation by experiments on artificial lipid bilayers.

Table 2. Comparison of the ratio, free iridals (FI):iridal esters (IE), after incorporation assay of iridals in microsomal suspension of different origins

	FI:IE		
	Control*	Microsomal pellet	Supernatant
Fenugreek	4.29	6.35	1.90
Avocado 1	2.95	4.90	0.62
Avocado 2	3.55	5.51	0.79
Rat liver	3.53	5.33	0.76

*Control, indicates the ratio FI:IE of the suspension before repelleting. Experiments were carried out once for each kind of microsome.

EXPERIMENTAL

Material. Iris germanica L. and *Trigonella foenum graecum* L. (fenugreek) were cultivated in the garden of the University. Avocado pears (*Persea americana* Mill.) were obtained from local markets. Male wistar rats were purchased from Ifa-Credo (Lyon-France).

Microsomal preparation. All procedures were carried out at 4°. *Iris* rhizomes (*ca* 100 g), fenugreek seedlings or avocado fruit pulp, were treated according to ref. [22]. *Iris* microsomes were prepd either immediately or following induction of cytochrome P-450 by $MnCl_2$ according to ref. [23]. Rat liver microsomes were prepd as described in ref. [24]. Microsomal pellets were resuspended in 0.1 M Na-Pi buffer (pH 7.4), 30% glycerol with 1.5 mM β-mercaptoethanol and stored at − 20°.

Protein assays. Microsomal protein content was determined with bicinchoninic acid reagent from Pierce (U.S.A.).

Characterization of microsomal fractions. Microsomal frs were characterized by the presence of P-450 which was estimated from its CO-difference spectrum according to

ref. [25]. This membrane-linked monooxygenase (well-known in animals) has been detected in microsomes from *I. germanica*, as well as in a wide variety of plant species [26]. We used this enzyme not only because it is easily identified by its specific visible spectra, but also because it is able to hydroxylate plant lipophilic substrates [27, 28]. Since *Iris* rhizomes contain several hydroxylated derivatives of iridals, it is reasonable to hypothesize that P-450 should be involved in iridal biosynthesis pathways. The P-450 content of the different microsome samples expressed in nmol mg^{-1} of protein was for avocado 0.290, fenugreek 0.040, untreated iris 0.015, $MnCl_2$-induced iris 0.075 and rat liver 0.680.

Sterol assays. Extraction of microsomal sterols was achieved according to ref. [29] and analysed by GC according to ref. [30].

Iridal analysis. Crude lipid extracts were analysed by HPLC as previously described [6].

In vitro *addition of iridals to microsomal suspensions.* Different mixts of FI in 70% EtOH (20 μl) and IE as oil (*ca* 15–20 μl) were added to re-suspended microsomes (6 ml) with 20 mM Na-Pi buffer (pH 7.4). The mixt. was then treated as indicated in ref. [21]. Controls consisted of 0.7 ml of each post-sonicated suspension. The remaining 5.3 ml were used for re-pelleting microsomes at 105 000 *g*. Iridal content was determined in control, pellet and supernatant frs.

Acknowledgements—Drs C. Cerdon and J. C. Baccou for useful discussions and technical assistance are gratefully acknowledged. The authors wish to thank Dr. F.-J. Marner for his help in the determination of iridals.

REFERENCES

1. Marner, F.-J., Krick, W., Gellrich, B. and Jaenicke, L. (1982) *J. Org. Chem.* **47**, 2531.
2. Krick, W., Marner, F.-J. and Jaenicke, L. (1983) *Z. Naturforsch* **38c**, 179.
3. Marner, F.-J., Littek, A., Arold, R., Seferiadis, K. and Jaenicke, L. (1990) *Liebigs Ann. Chem.* 563.
4. Littek, A. and Marner, F.-J. (1991) *Helv. Chim. Acta.* **74**, 2035.
5. Abe, F., Chen, R. F. and Yamauchi, T. (1991) *Phytochemistry* **30**, 3379.
6. Bonfils, J.-P., Sauvaire, Y., Baissac, Y. and Marner, F.-J. (1994) *Phytochemistry* **37**, 701.
7. Marner, F.-J., Ritzdorf, I. and Johnen, G. (1993) *Phytochemistry* **33**, 573.
8. Dupéron, R., Brillard, M. and Dupéron, P. (1972) *C. R. Acad. Sci. Paris* **274**, 2321.
9. Norberg, P. and Liljenberg, C. (1991) *Plant Physiol.* **96**, 1136.
10. Galliard, T. (1968) *Phytochemistry* **7**, 1907.
11. Galliard, T. (1968) *Phytochemistry* **7**, 1915.
12. Douglas, T. J. and Paleg, L. G. (1981) *J. Exp. Botany* **32**, 499.
13. Svenningsson, H. and Liljenberg, C. (1986) *Physiol. Plant.* **68**, 53.
14. Navari-Izzo, F., Quartacci, M. F. and Izzo, R. (1989) *J. Exp. Botany* **40**, 675.
15. Navari-Izzo, F., Vangioni, N. and Quartacci, M. F. (1990) *Phytochemistry* **29**, 2119.
16. Dupéron, R. and Dupéron, P. (1989) *C. R. Acad. Sci. Paris* **308**, 31.
17. Marner, F.-J. and Kerp, B. (1992) *Z. Naturforsch.* **47c**, 21.
18. Grunwald, C. (1980) *Enc. Plant Physiol.* **8**, 221.
19. Kemp, R. J. and Mercer, E. I. (1968) *Biochem. J.* **110**, 119.
20. Bonfils, C., Larroque, C., Bonfils, J.-P. and Charasse, S. (1994) Xth International Symposium on Microsomes and Drug Oxidations, Toronto, Canada.
21. Cooke, D. T., Ros, R., Burden, R. S. and James, C. S. (1993) *Physiol. Plant.* **88**, 397.
22. Gabriac, B., Werck-Reichhart, D., Teutsch, H. and Durst, F. (1991) *Arch. Biochem. Biophys.* **288**, 302.
23. Reichhart, D., Salaün, J.-P., Benveniste, I. and Durst, F. (1980) *Plant Physiol.* **66**, 600.
24. Larroque, C., Lange, R., Maurel, P., Langlois, R. and Van Lier, J. (1989) *J. Steroid Biochem.* **33**, 277.
25. Omura, T. and Sato, R. (1964) *J. Biol. Chem.* **239**, 2370.
26. Rich, P. R. and Bendall, D. S. (1975) *Eur. J. Biochem.* **55**, 333.
27. Pinot, F., Salaün, J.-P., Bosch, H., Lesot, A., Mioskowski, C. and Durst, F. (1992) *Biochem. Biophys. Res. Commun.* **184**, 183.
28. Funk, C. and Croteau, R. (1993) *Plant Physiol.* **101**, 1231.
29. Grandmougin, A. (1989) Ph.D. Thesis, Université Strasbourg, France.
30. Brenac, P. (1993) Ph.D. Thesis, Université Montpellier II, France.