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CITRUS BITTER PRINCIPLES—V.

BOTANICAL DISTRIBUTION AND CHEMOTAXONOMY IN THE RUTACEAE

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Abstract—Examination of the limonoids in extracts from seeds of twenty-six Citrus species and hybrids showed limonin to be present in every case, and, with two exceptions, in greater amounts than obacunone and deacetylnomilin. Limonoids have also been shown to occur in the related genera, Poncirus, Microcitrus and Fortunella. New results are presented on the distribution of limonoids in other genera of the Rutaceae, i.e. Casimiroa, Evodia and Caladendrum. The value in chemical taxonomy of these data when combined with previous results is discussed. Comparison is made with limonoids occurring in the Meliaceae, and limonoid degradation products, the simaroubolides, occurring in the Simaroubaceae. In general, the distribution pattern of limonoids and simaroubolides in the Rutaceae, Meliaceae and Simaroubaceae correlates very well with the botanical divisions based upon orthodox taxonomy. Isopimpinellin has been isolated from seeds of Aeglopsis chevalieri Swing. and Bergapten from Cneoridium dumosum (Nutt.) f. Xanthotoxol and allo-imperatorin have been isolated from seeds of Poncirus trifoliata.

INTRODUCTION

THE report¹ of the structure determination of limonin (I) in 1960 opened the way for assignment of structures to similar complex C_{26} terpenoids in the Rutaceae and Meliaceae. It was suggested at that time that limonin arose as an oxidative degradation product of a tetracyclic triterpene of the butyrospermol type and that obacunone (II) and nomilin (IIIa) were precursors in the final stages of the biogenesis of limonin. Subsequently, the isolation of additional formal intermediates in the biogenesis of limonin has been reported from the closely related family, Meliaceae.²

There have been many reports³ of limonoid compounds, especially limonin itself, from members of the genus *Citrus*. This certainly is partly due to the ease with which limonin

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- ¹ D. ARIGONI, D. H. R. BARTON, E. J. COREY, O. JEGER, L. CAGLIOTA, SUKH DEV, P. G. FERRINI, E. R. GLAZIER, A. MELERA, S. K. PRADHAN, K. SCHAFFNER, S. STERNHELL, J. F. TEMPLETON and S. TOBINAGA, *Experientia* 16, 41 (1960); S. ARNOTT, A. W. DAVIE, J. M. ROBERTSON, G. A. SIM and D. G. WATSON, *Experientia* 16, 49 (1960); D. H. R. BARTON, S. K. PRADHAN, S. STERNHELL and J. F. TEMPLETON, *J. Chem. Soc.*, 255 (1961)
- ² C. W. L. BEVAN, A. H. REES and D. A. H. TAYLOR, J. Chem. Soc., 983 (1963) and previous papers in this series; C. W. L. BEVAN, T. G. HALSALL, M. N. NWAJI and D. A. H. TAYLOR, J. Chem. Soc., 768 (1962); R. HODGES, S. G. MCGEACHIN and R. A. RAPHAEL, J. Chem. Soc., 2515 (1963); J. R. HOUSLEY, F. E. KING, T. J. KING and P. R. TAYLOR, J. Chem. Soc., 5095 (1962).
- ³ For a summary of earlier references to limonin see M. S. SCHECHTER and H. L. HALLER, J. Am. Chem. Soc. 62, 1307 (1940); L. BRACHVOGEL, Arch. Pharm. 285, 57 (1952); A. MELERA, K. SCHAFFNER, D. ARIGONI and O. JEGER, Helv. Chim. Acta 40, 1420 (1957); see also T. R. SESHADRI, Proc. Indian Acad. Sci. 18A, 201 (1943); Chem. Abstr. 39, 1438 (1945); D. NOMURA, Hakko Kogaku Zasshi 30, 199 (1952); Chem. Abstr. 47, 791 (1953); T. R. SESHADRI and J. VEERARAGHAVIAH, Proc. Indian Acad. Sci. 11A, 505 (1940); Chem. Abstr. 34, 8090 (1940); A. MOOKERJEE, J. Indian Chem. Soc. 17, 593 (1940); Chem. Abstr. 36, 4283 (1942); C. INAGAKI, Nat. Sci. Rep. Ochanimizu Univ. Tokyo, 2, 133 (1951); Chem. Abstr. 47, 5038 (1953); L. R. Row and G. P. SASTRY, J. Sci. Ind. Res. (India), 21B, 343 (1962); Chem. Abstr. 57, 16728 (1962).

crystallizes. As part of a program of studies on citrus bitter principles, it was of interest to examine their distribution and to see if further biogenetic intermediates of limonin could be detected or if more advanced conversion products of limonin could be found in the Rutaceae. Naturally occurring limonin diosphenol $(IV)^4$ is such an example of a further oxidation product of limonin. This survey for new limonoids has resulted in the isolation of deacetyl-nomilin (IIIb),⁵ deoxylimonin⁵ and ichangin (V).⁶ Furthermore, a study of this kind might establish the value of limonoids as chemical taxonomic markers among *Citrus* and its relatives. This may be of some value in view of the confused state of the taxonomy in these groups.



RESULTS

Citrus Species

As described previously,⁵ thin-layer chromatography (TLC) is a useful tool for monitoring limonoids when used with Ehrlich's reagent, a highly selective spray reagent for furanoid

- 4 Y. HIROSE, Chem. Pharm. Bull. (Tokyo) 11, 535 (1963).
- ⁵ D. L. DREYER, J. Org. Chem. 30, 749 (1965).
- ⁶ D. L. DREYER, Abstr. Papers 149th Am. Chem. Soc. Meet., 16P (1965).

compounds. Some coumarins, especially psoralens, give positive tests with this reagent, but their colors differ from those of limonoids. By this means, the seeds of thirteen *Citrus* species and thirteen *Citrus* hybrids have been surveyed for their limonoid composition (Table 1).⁷

	Obacunone (II)	Limonin ^a (I)	Deacetyl- nomilin (IIIb ^b)
C. medica Linn.	+	+	
C. limon (Linn) Burm.f. ^b	+	+	_
C. aurantifolia (Christm.) Swing.	-	+	+
C. aurantium Linn. ^c	-	+	+ "
C. sinensis (Linn.) Osbeck ^b	+	+	+*
C. reticulata Blanco	+	+	+
C. grandis (Linn.) Osbeck	+"	+	+ "
C. paradisi Mact.	+*	+	+"
C. indica Tan.	+	+	+
C. tachibana (Mak.) Tan.	+	+	+
C. ichangensis Swing.	+	+	+
C. macroptera	-	+	+
C. hystrix D. C.		+	+
	In Citrus Hybrids		
Citrumelo			
P. trifollata × C. paradisi	-	+	+
Limequat			,
C. aurantijona × r. japonica		Ť	+
Carrizo citrange			
C. sinenses × P. trifoliata	-	+	+
Calamondin			
C. reticulata × F. sp.	_	+	—
Shekwashi mandarin			
Probably C. tachibana \times C. reticulata	-	+	-
Altoona tangor			
C. reticulata \times C. sinensis	+	+	+
Yuzu ^c			
C , ichangensis $\times C$, reticulata	+	+	+ "
Ichang lemon ^{b, c}	•		•
C ichangensis × C grandis?	+ a	1	a
C. Ichangensis ~ C. granais:	т	т	т
C. Knarna ^{ste} Dessible lemen en lemen bebeid	1		. 4
Possible lemon or lemon hybrid	+	+	+-
C. pennivesiculata ^c	+	+	+
Canaliculata	+	+	+
Rangpur lime			
C. reticulata × ?	+	+	-
C. macrophylla			
possibly C, celebica \times C. grandis	+	+	_
P	·	•	

TABLE 1. DISTRIBUTION OF LIMONOIDS IN Citrus SPECIES⁷

^a Isolated and characterized by m.p. and i.r. spectrum.

^b Nomilin(IIIa) isolated in this study.

^c TLC shows further unidentified limonoids.

⁷ The botanical classification followed in this discussion is largely that of Swingle; W. T. SWINGLE, *The Citrus Industry* (Edited by H. H. WEBBER and L. D. BATCHELER), Vol. I, Ch. IV. University of California Press, Berkeley (1943).

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In each case, limonin was isolated from the extracts by crystallization and characterized by m.p. and its characteristic i.r. spectrum. The identification of the other components was made, except where noted, on their R_f only. However, in view of the high specificity of the spray reagent, the assignments are regarded as quite certain. Obacunone ran well ahead of limonin. Limonin and nomilin could not be resolved with all brands of silicic acid. Because of this uncertainty no attempt has been made to distinguish nomilin by TLC in this work. Deacetylnomilin (IIIb) ran about halfway between limonin and the origin.

With certain rare species, only limited amounts of fruit from one tree were available. In these cases the results obtained are due to only one sample and the data reported in the table must be judged with this limitation in mind. However, the results from these sample limitations may be more reliable than they appear. For example, many different batches of lemon seeds, of different varieties and from different localities were processed without detection of deacetylnomilin (IIIb).

It is apparent from inspection of Table 1 that limonin (I) is the dominant and characteristic limonoid in *Citrus*. It occurred in major amounts except in two cases. Deacetylnomilin (IIIb) was the major limonoid in ichang lemon seeds and in some samples of *C. kharna* seeds.

All the Citrus species investigated exhibited essentially the same pattern of limonoids with the exception of C. ichangensis and its hybrids. These plants show a remarkable ability to accumulate relatively large amounts of limonin intermediates. They showed four additional unknown minor limonoids, two less polar than limonin and two more polar. One of these more polar materials, ichangin (V), has been isolated and its structure reported.⁶ C. macroptera also showed two minor spots, one nonpolar and one polar relative to limonin. C. pennivesiculata showed two limonoid type spots in the obacunone region and some very polar spots which appear to be acidic limonoids.^{*}

Citrus Relatives

This study was extended to several close *Citrus* relatives. The seeds of the monotypic genus, *Poncirus*, were strikingly similar to those of *Citrus* in their limonoid content.^{5, 8} Seeds of *P. trifoliata* contained obacunone (II), limonin (I) and deacetylnomilin (IIIb). However, unlike *Citrus* seeds, which have a high oil content but few if any coumarins, the seeds of *P. trifoliata* have a low oil content and a high coumarin content, composed mainly of imperatorin (X),^{5, 8} bergapten⁵ and smaller amounts of xanthotoxol and alloimperatorin (VII). Nomilin (IIIa) has also been isolated from *P. trifoliata* seed extracts by chromatography on alumina.

The alloimperatorin (VII)⁹ was converted to a monoacetate (VIII) under mild conditions and to a monomethyl ether (IX) with diazomethane. The NMR spectra of these coumarins (VII to IX) were consistent with the assigned structures and indicated the presence of an isopentenyl group which could not be hydrolyzed under the usual mild conditions.¹⁰ Alloimperatorin has previously been reported as a constituent of *Aegle marmelos* Correa¹¹ and was prepared by the thermal rearrangement of imperatorin (VIII).

Microcitrus is a primitive genus related to *Citrus.*⁷ The seed extracts of *M. australasica* var. *sanguinea* (F. M. Bail.) Swing. were examined by TLC. The extracts contained two

* Only β -situated could be isolated from the nonsaponifiable fraction of the hexane extracts of navel orange seeds (*C. sinensis*) and grapefruit seeds (*C. paradisi*).

⁸ G. K. NIKONOV and M. M. MOLODOZHNIKOV, *Medskaya Prom. SSSR* 18, 24 (1964); *Chem. Abstr.* 61, 14466 (1964).

⁹ E. SPAETH and H. HOLZEN, Ber. Deut. Chem. Ges. 66B, 1137 (1933).

¹⁰ See, for example, W. L. STANLEY and S. H. VANNIER, J. Am. Chem. Soc. 79, 3488 (1957).

¹¹ S. K. SAHA and A. CHATTERJEE, J. Indian Chem. Soc. 34, 228 (1957); Chem. Abstr. 51, 16730 (1957).

limonoid components whose R_f 's corresponded to limonin and deacetylnomilin, but the amount of seeds available was too small to allow isolation of either component for complete characterization.



Both limonin (I) and obacunone (II) could be detected in seeds of Fortunella margeritia. The seed extracts resembled those of Citrus with a high oil content and little evidence for coumarins. Limonoids could not be detected in extracts of the whole fruit* of the monotypic genus, Eremocitrus (E. glauca). These extracts contained a number of flavonoids and coumarins. Extracts of the whole fruit of Severinia buxifolia (Poir.) Ten. and seeds of Glycosmis citrifolia gave no evidence for limonoids. Extracts of seeds of Aeglopsis chevalieri Swing. were also devoid of limonoids, but did contain 5,8-dimethoxypsoralen (isopimpinellin).

Other Members of the Rutaceae

Limonoids have previously been found in six widely distributed genera of the Rutaceae: Casimiroa,^{12, 13} Phellodendron,^{14, 15} Evodia,^{4, 14, 16} Vepris,¹⁷ Dictamnus^{14, 18} and Citrus.³ During the present studies some of the species from which limonoids were previously reported have been reinvestigated. Table 2 summarizes the data on the distribution of limonoids in genera of the Rutaceae.

Constituents of the species Casimiroa edulis Llave et Lex have been intensively studied by Sondheimer and coworkers.¹⁹ Obacunone was reported to occur in the seeds of this species.^{12, 13} Reinvestigation of these seed extracts confirmed the presence of obacunone (II) and established the presence of nomilin (IIIa). Careful TLC gave no evidence for the presence

* The sample was an *Eremocitrus* \times *Citrus* hybrid. The whole fruit was extracted because of the small seed size.

- ¹² F. A. KINCL, J. ROMO, G. ROSENKRANZ and F. SONDHEIMER, J. Chem. Soc. 4163 (1956).
- ¹³ F. SONDHEIMER, A. MEISELS and F. A. KINCL, J. Org. Chem. 24, 870 (1959).
- ¹⁴ T. TOKOROYAMA, Kashi 79, 314 (1958).
- ¹⁵ T. KUBOTA and T. TOKOROYAMA, Chem. & Ind. (London), 1298 (1957); A. FUJITA and K. WADA, J. Pharm. Soc. Japan 51, 506 (1931); Chem. Abstr. 25, 4870 (1931); T. KAKU and H. RI, J. Pharm. Soc. Japan 55, 222 (1935); Chem. Abstr. 31, 6643 (1937).
- ¹⁶ S. MAEDA, J. Pharm. Soc. Japan 55, 531 (1935); Chem. Abstr. 29, 5831 (1935); A. FUJITA and M. AKATSUKA, J. Pharm. Soc. Japan 69, 322 (1949); Chem. Abstr. 44, 1954 (1950).
- 17 T. R. GOVINDACHARI, B. S. JOSHI and V. M. SUNDARANAJAN, Tetrahedron 20, 2985 (1964).
- ¹⁸ H. THOMS, Ber. Deut. Pharm. Ges. 33, 68 (1923); Chem. Abstr. 17, 2583 (1923); T. KAKU and H. RI, J. Pharm. Soc. Japan 55, 219 (1935); Chem. Abstr. 31, 6642 (1937).
- ¹⁹ R. MECHOULAM, F. SONDHEIMER, A. MELERA and F. A. KINCL, J. Am. Chem. Soc. 83, 2022 (1961) and previous papers in this series.

of limonin but did show the presence of three to four additional polar limonoids. One of the more polar materials has been isolated. The NMR spectrum of this compound showed the presence of five C-methyl groups, indicating that it is related to obacunone rather than to the more highly oxidized limonin derivatives which contain only four C-methyl groups.

Subfamily	Aurantioideae			Toddalioideae		Rutoideac				
Genus	Citrus .	Poncirus	Fortunella	Microcitrus	Phellodendron	Casimiroa	Vepris	Calodendrum .	Evodia	Dictamnus
Deacetylnomilin (IIIb)	×	×	· · · · ·							
Nomilin (IIIa)	x	×				x				
Obacunone (II)	×	×	×		×	×				×
Limonin (I)	×	×	×	x	×			×	×	×
Limonin diosphenol (IV)								×	×	
Deoxylimonin	×									
Ichangin (V)	×									
Veprisone							×			
Rutaevin (VI)								×	×	×b

TABLE 2. DISTRIBUTION OF LIMONOIDS^a IN GENERA OF THE RUTACEAE

^a Limonexic acid (Ref. 1) has not been considered since it is probably an artifact arising from limonin.

^b The physical and chemical properties of dictamnolide (Ref. 16) suggest that it is identical with rutaevin.

TLC of extracts from *Casimiroa tetrameria* Millsp. seeds clearly showed the presence of obacunone and nomilin but lack of material has precluded isolation of either component.

Obacunone has been isolated from three of the nine known *Phellodendron* species.^{14, 15} Re-examination of the limonoids in the bark of *P. amurense* Rupr. by TLC showed *only two* components, obacunone and limonin, both of which were isolated.

Table 3 summarizes the limonoid distribution in *Evodia* which is a large genus of about 120 species. Limonin (I), limonin diosphenol (IV) and rutaevin (VI) have been reported to occur in *E. rutaecarpa* Benth. and Hook.^{4, 14, 16} Re-examination of *E. rutaecarpa* seed

	Limonin (I)	Limonin diosphenol (IV)	Rutaevin (VI)
E. danielli hensel ^a	×	X	
E. rutaecarpa Benth. and Hook	×	×	×
E. hupenhensis ^b	×	×	×
E. meliaefolia ^c	×		

TABLE 3. DISTRIBUTION OF LIMONOIDS IN Evodia SPECIES

" These data taken from Ref. 14.

^b This species contains a second unidentified limonoid.

^c Contains a fourth, polar limonoid.

extracts by TLC showed only these three limonoids. The NMR spectrum of rutaevin showed that it is closely related structurally to limonin.* This study has been extended to several other *Evodia* species. Only limonin could be detected in the heartwood of *E. hupenhensis*. The seeds of this species contained limonin as the major component and lesser amounts of rutaevin. TLC indicated the presence of an additional component, and the TLC pattern was identical with that of *E. rutaecarpa*. Seeds of *E. meliaefolia* Benth. showed only two limonoid spots on TLC. One of the components was isolated and proved to be limonin. The identity of the other more polar material is uncertain because of small sample size.

The seeds of *Calodendron capensis* (Cape chestnut) showed the same limonoid pattern as *E. rutaecarpa* and *E. hupenhensis*. The isolation of pure rutaevin (VI) is much more practical from this plant than from *E. rutaecarpa*. In the latter it is often contaminated with u.v. absorbing impurities.

Members of three genera of the Rutaceae, *Thamnosma montana* Torr. and Frem.,²⁰ *Ptelea trifoliata*²¹ and *Cneoridium dumosum* (Nutt.) f., native to the far southwestern United States, have also been surveyed for limonoids with negative results. Bergapten was isolated from the aerial parts of *Cneoridium dumosum* (Nutt.) f.

DISCUSSION

With regard to their seed content, *Citrus* species constitute a remarkably homogeneous group. The seeds are characterized by a high oil content, few if any coumarins and a fairly uniform limonoid content. The limonoid content (Table 1) is dominated by the presence of limonin in major amounts with the other three limonoids present in small and somewhat variable amounts. The same situation occurs in the intra- and inter-genetic hybrids of *Citrus*. It is noteworthy that some members of the subgenus *Papeda* (for example, ichang lemon, yuzu and *C. macroptera*) appear to show more complexity in their composition and contain several additional minor limonoid constituents. On the other hand, *C. hystrix*, also a member of this subgenus, contained only II and IIIa. The greatest number of different limonoid compounds have been reported from *Citrus* (Table 2), certainly due to investigations motivated by its economic importance.

The available data make possible a few generalizations on the distribution of limonoids in the Rutaceae. First, when limonoids occur in one member of a genus then it appears that all species of that genus contain limonoids. Secondly, the oxidation state of all the limonoids is about the same level for any given species or genus. For example, the limonoids isolated from *Evodia* species are all of a high oxidation level, being composed of limonin or limonin derivatives. *Casimiroa*, on the other hand, appears to contain only limonoids of a lower oxidation level, all being related to obacunone. *Citrus* and *Citrus* relatives appear to occupy an intermediate position in this regard as they contain compounds of both the obacunone and limonin oxidation level, but none have yet been isolated of a higher oxidation level than limonin.

The co-occurrence of nomilin with obacunone in widely distributed species of the Rutaceae, for example, *Casimiroa* and *Citrus* adds strong circumstantial evidence for the previously

^{*} The structure of rutaevin will be reported shortly.

²⁰ Three coumarins have been reported from this plant. E. L. BENNETT and J. BONNER, Am. J. Botany 40, 29 (1953).

²¹ The isolation of alkaloids has been reported from P. trifoliata. V. I. FROLOVA, A. D. KUZOVKOV and P. N. KIBATCHICH, Zh. Obshch. Khim. 34, 3499 (1964); Chem. Abstr. 62, 2800 (1965); M. KOWALSKA, Acta Polon. Pharm. 20, 458 (1963); Chem. Abstr. 62, 6797 (1965).

proposed⁵ role of nomilin as a biogenetic precusor of obacunone even though determined attempts to detect nomilin in *Phellodendron* species were unsuccessful.

It may be instructive to consider the possible value of the limonoids from a chemical taxonomic viewpoint. The reader is directed to reviews by Price²² and Ritchie²³ for most useful discussions of chemical taxonomy in the Rutaceae based on the alkaloids and coumarins. The Rutaceae is divided into seven subfamilies (Table 4).²⁴ Three of these subfamilies are monogeneric and the bulk of the genera and species are confined to three of the subfamilies, Rutoideae, Toddalioideae and Aurantioideae. The data presently available indicate that genera containing limonoids are not confined to any particular subfamily of the Rutaceae but are rather evenly spread throughout the family at a low frequency of occurrence. Because the Rutaceae has been a fairly well investigated family, and considering the ease with which most limonoids can be isolated and crystallized, the low frequency of occurrence may be a valid conclusion.





" The total number of species in each genus follow the genus name.

^b The position of flindissol (Ref. 25), a metabolite of Flindersia, is somewhat ambiguous, for, if it is a member of the limonoids it is very far down on the biogenetic ladder. The main reasons for accepting flindissol as a member of the limonoids are, (a) it has the prerequisite stereochemistry for a limonoid precursor and (b) it has a tetrahydrofuran ring correctly located for conversion to the C-17 furan ring of the limonoids. It is interesting that this borderline limonoid belongs also to the subfamily around which has centered so much difference of opinion as to its botanical relationship to the Rutaceae.²²

Although limonoids occur in each of the major subfamilies of the Rutaceae, there is some bunching of the distribution in the Toddalioideae and Aurantioideae. In the former subfamily, *Casimiroa* and *Vepris* are two very closely related genera. In the Aurantioideae, limonoids have only been reported in *Citrus* and its close relatives. This study has not detected limonoids in *Eremocitrus*. The classification of *Eremocitrus* on morphological grounds between limonoid possessing *Microcitrus* and *Poncirus* would certainly lead to the prediction of limonoids in this genus. Obviously, further investigation of *Eremocitrus* with greater amounts of material is warranted.

The limonoids (as opposed to other possible taxonomic markers) in the Rutaceae are of special interest in chemotaxonomy because they are largely built up in a single sequence of

²² J. R. PRICE, in *Chemical Plant Taxonomy* (Edited by T. SWAIN) Chap. 15. Academic Press, London (1963). ²³ E. RITCHIE, *Rev. Pure Appl. Chem.* 14, 47 (1964).

²⁴ A. ENGLER and K. PRANTL, Die Naturlichen Pflanzenfamilien (2nd Ed.) Vol. 19a, p. 187. Englemann, Leipzig (1931).

²⁵ A. J. BIRCH, D. J. COLLINS, S. MUHAMMAD and J. P. TURNBULL, J. Chem. Soc. 2762 (1963).

steps. Added information is thus available for classifying genera and species by the number of steps operating in any particular case. In particular, Casimiroa and Vepris (on the basis of the limited data available) contain only obacunone derivatives (Table 2) and are thus set apart by their inability to effect oxidative ring closure on to the C-19 methyl group. This is the step that leads from obacunone (II) to limonin (I) derivatives. It is of interest that these two genera are very closely related on classical taxonomic grounds. Even Phellodendron, the other limonoid possessing genus of this subfamily, shows less oxidative ability at C-19 than genera of the other subfamilies since limonin (I) occurs only in very small amounts relative to obacunone (II) in this genus. In contrast to genera of the subfamily Toddalioideae, limonoid producing genera of the Rutoideae are very efficient C-19 oxidizers producing rutaevin and limonin diosphenol (IV) which are at a higher oxidation level than limonin. All limonoid producing genera of the Aurantioideae appear to be very uniform and consistent in their limonoid producing ability and to be intermediate between the Rutoideae and Toddalioideae in their oxidizing ability.* One can thus rank the subfamilies in order of their increasing ability to produce more highly oxidized limonoids; i.e. Flindersioideae, Toddalioideae, Aurantioideae and Rutoideae. Limonoids thus seem to be of little value as taxonomic markers on a species level but are perhaps useful on a generic level.

Price²² has concluded, on the basis of the distribution of alkaloids and coumarins in the Rutaceae, that the major subfamilies constitute a highly homogeneous group. He points out that this is contrary to botanical considerations and that phylogenetically the Rutaceae is a complex family. The subfamilies of the Rutaceae are anatomically well defined and separated from one another. Price's conclusions²² are supported by the botanically uniform distribution of limonoids throughout the Rutaceae. The uniformity is further emphasized by the fact that structural variation of those limonoids that occur in the Rutaceae are rather slight (excepting flindissol²⁵ which appears to occur only in a further removed botanical group). These compounds (Table 2) constitute a closely related group which differ in structure largely in the A-ring. The homogeneity of the Rutaceae is attested to by the slight structural variation in the limonoids while the clear definition between subfamilies is attested to by the uniform difference in oxidation levels of their limonoids.

Cedrelioidea	Swietenioideae	Melioideae
Cedrela (45)	Khaya (8) Entandrophragma (15)	Carapa (15) Xylocarpus (7)
	Swietenia (7)	Trichilia (230) Melia (9)
		Guarea (170)

Table 5. Distribution of limonoids by subfamilies and genera in the meliaceae a

^a The total number of species in each genus follows the genus name.

Limonoids appear also to be rather evenly distributed throughout the closely related family, Meliaceae. Limonoids have been found in genera² belonging to each of the three subfamilies of the Meliaceae (Table 5)²⁶ but in this case there seems to be no general cor-

^{*} A very interesting question is raised by these observations: Are these differences between subfamilies due to mutations causing blockage of the biosynthetic route or are they due to evolutionary differences?

²⁶ A. ENGLER and K. PRANTL, Die Natürlichen Pflanzenfamilien (2nd Ed.) Vol. 19b, p. 1. Englemann, Leipzig (1931).

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relation between the limonoid structure and the botanical distribution within the family. Also, as opposed to the Rutaceae, some species of limonoid possessing genera in the Meliaceae appear not to contain limonoids.²⁷ The known compounds of this series in the Meliaceae show, in general, much wider structural variation than those in the Rutaceae. These varients range from members whose position is fairly low on the biogenetic ladder, such as, gedunin,² cedrelone² and anthothecol,² to those with extensive structural alterations, for example, andirobin,²⁸ swietenine,²⁹ nimbin,³⁰ mexicanolide³¹ and methyl angolensate.³²

The biogenetic relationship of the limonoids with the simaroubolides has previously been pointed out.³³ The close structural relationship of limonoids and simaroubolides parallels the close taxonomic relationship of the Rutaceae and Meliaceae with the Simaroubaceae.^{7, 33} Simaroubolides³⁴ are compounds which have undergone the most extensive alterations to the basic C_{26} carbon skeleton and as such are the most highly advanced biogenetically of this class of materials. While simaroubolides are very closely related to limonoids by structural analogy and botanical origin they are also, on the basis of the available data, clearly a group set apart from limonoids. Thus far there has been no report of co-occurrence of the two types of bitter principles in the same botanical group.

EXPERIMENTAL

Citrus, Microcitrus and Fortunella Seeds

Dried and ground seeds were defatted with hexane and then extracted with acetone. The residue remaining after removal of solvent from the acetone extracts was crystallized from methylene chloride-isopropanol to give limonin. Limonoids in the mother liquors could be concentrated by washing the residue with hexane to remove further traces of oils. The residue could also be filtered through a short column of acid-washed alumina with chloroform to remove polar impurities. These eluants could then be used for spotting to give cleaner thin-layer chromatograms.

Poncirus trifoliata Seeds

The experimental procedure for working up extracts of *Poncirus* seeds as well as isolating deacetylnomilin, imperatorin, and bergapten has been previously reported.⁵ Further chromatography on acid washed alumina gave nomilin. Xanthotoxol was isolated from the

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carbonate washings of the mother liquors of the acetone extracts. It was identical in all respects with a sample prepared from imperatorin.¹⁰ NMR 473(d) J=10 H-4, 467(d) J=2 H-7, 435(s) H-5, 410(d) J=2 H-6, 376(d) J=10 H-3, c/s (deuteriochloroform-dimethyl-sulfoxide)*.

Alloimperatorin (VII)⁹

The light petrol soluble material from the acetone extracts of *P. trifoliate* seeds from which the bulk of the bitter principles had been removed by crystallization, was allowed to stand for about 4 months during which time crystalline material was deposited. Sublimation and recrystallization from ethanol and ethyl acetate or acetic acid gave alloimperatorin (VII) m.p. 229–232°; lit.⁹ m.p. 233°; λ_{max} 221 (21700), 244(9200), 252(9300), 268(11700), 273(11700), 317(7700) m μ (95% ethanol); i.r. ν 3330 (hydroxyl), 1730, 1596 cm⁻¹ (Nujol); NMR 493(d) J=10 H-4, 487(d) J=2 H-7, 427(d) J=2 H-6, 389(d) J=10 H-3, 312(t) J=7 vinyl, 224(d) J=6 allyl methylene, 111,101(s) γ -methyls c/s (dimethyl sulfoxide). (Found: C, 71·1; H, 5·27. Calc. for C₁₆H₁₄O₄: C, 71·1; H, 5·22%.) The natural material was identical in all respects with a synthetic sample prepared from imperatorin as described below.

Alloimperatorin Acetate (VIII)

Acetylation was carried out with acetic anhydride-pyridine by allowing the solution to stand at room temperature. The acetate was recrystallized from ethanol, m.p. 135-136°; λ_{max} 218, 249, 263, 302 m μ (95% ethanol); i.r. ν 1785, 1731, 1595 cm⁻¹ (Nujol); NMR 479(d) J = 10 H-4, 460(d) J = 2 H-7, 413(d) J = 2 H-6, 379(d) J = 10 H-3, 309(t) J = 6 vinyl, 221(d) J = 6 allyl methylene, 148(s) acetate, 110, 102(s) γ -methyls c/s (deuteriochloroform). (Found: C, 69·3; H, 5·17. Calc. for C₁₈H₁₆O₅. C, 69·22; H, 5·16%). This sample was identical in all respects with a sample of VIII prepared in the same manner from synthetic VII.

Alloimperatorin Methyl Ether (IX)⁹

Natural VII was methylated with diazomethane and the product crystallized from dilute ethanol, m.p. 104–105°; λ_{max} 220(20400), ~244(12500), 250(13500), 266(12100), 308(8400) m μ (95% ethanol); i.r. ν 1725, 1590 cm⁻¹ (Nujol); NMR 480(d) J=10 H-4, 462(d) J=2 H-7, 413(d) J=2 H-6, 383(d) J=10 H-3, 310(t) J=7 vinyl, 255(s) methoxy, 223(d) J=7 allyl methylene, 111, 103(s) γ -methyls c/s (deuteriochloroform). Attempted hydrolysis of IX with conc. sulfuric acid in glacial acetic acid and warming on a steam bath¹⁰ resulted in recovery of starting material.

Rearrangement of Imperatorin (X) to Alloimperatorin (VII)

A solution of imperatorin in dibutyl phthalate was refluxed gently for 45 min. The solution was cooled and the product separated on standing. Alternatively the reaction mixture was diluted with ether and extracted with 5% NaOH. The base extracts were acidified and the product collected. Recrystallization from methanol gave alloimperatorin (VII), m.p. 233-235°; u.v. and i.r. spectra were superimposable on those from the natural material.

Aeglopsis chevaleri Swing.

The hexane extracts of dried and ground seeds were concentrated and allowed to stand in a freezer for several days. Crystalline material which deposited was collected and re-

^{*} NMR spectra were obtained at 60 Mc/s and are given in c/s relative to internal tetramethylsilane. The relative areas of peaks were consistent with the assignments.

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crystallized from ethyl acetate-hexane; m.p. 146-147°, identical with an authentic sample of isopimpinellin prepared from xanthotoxin according to published procedures.³⁵ Infra red ν 1744, 1714, 1589 cm⁻¹ (Nujol); NMR 491(d) J = 10 H-4, 462(d) J = 2 H-7, 425(d) J = 2 H-6, 379(d) J = 10 H-3, 251, 252(s) methoxyls c/s (deuteriochloroform).

Casimiroa edulis Llave et Lex.

The seeds were extracted and the extracts worked up as described previously.¹² The fractions showing limonoids by TLC were chromatographed over acid washed alumina. The fractions from the column were monitored by TLC. Obacunone was eluted with benzene and nomilin with chloroform, m.p. 276-278°, from ethanol. Elution with 10% acetone in chloroform gave a further unidentified limonoid, m.p. 249-250°, from methanol. Identity of the obacunone and nomilin was established by comparison with authentic samples.

Phellodendron amurense Rupr.

The dried and ground bark was extracted with acetone. The solvent was removed and successive crops of obacunone were obtained by crystallization from ethanol. TLC showed only one additional polar limonoid present in the mother liquors. This proved to be limonin upon work-up of the mother liquors by chromatography over acid washed alumina.

Evodia meliaefolia Benth.

The seeds were ground and extracted with chloroform. Solvent was removed from the extracts and the residue was washed with hexane to remove oils. The hexane insoluble material was crystallized from ethanol to give limonin. The mother liquors showed a limonoid spot R_f 0.80 relative to that of limonin by TLC.

Evodia hupenhensis

The dried and ground heartwood was extracted with acetone and the acetone extracts worked up to give crystalline limonin. TLC examination of the mother liquors showed no other limonoids. Ground seeds of *E. hupenhensis* were extracted with acetone. Solvent was removed from the extracts and the residue was washed with hexane to remove oils. The residue was crystallized from chloroform-ethanol to give two crops of limonin. The mother liquors deposited rutaevin upon standing. TLC of the mother liquors also showed a spot corresponding in R_f to limonin diosphenol.

Cneoridium dumosum (Nutt.) f.

The whole aerial part of the plant, including fruit, collected in February near San Diego, California was ground and extracted with hexane. Upon standing the hexane extracts deposited bergapten which was identical in all respects with an authentic sample. Bergapten was also recovered upon work-up of the acetone extracts. TLC of the acetone mother liquors gave no evidence for limonoids.

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