Phytochemistry, 1972, Vol. 11, pp. 705 to 713. Pergamon Press. Printed in England.

DISTRIBUTION OF LIMONOIDS IN THE RUTACEAE*

David L. Dreyer, Michael V. Pickering and P. Cohan
Department of Chemistry, San Francisco State College, San Francisco, California, U.S.A.

(*Received* 1 *June* 1971)

Abstract—Chemical studies on *Choisya arizonica*, *C. mollis*, *C. ternata*, *Esenbeckia hartmanii*, *Hesperethusa crenulata*, *Eremocitrus glauca*, *Triphasia trifolia*, *Coleonema album* and *Pamburus missionis* are described with special reference to the limonoid bitter principles. The distribution of limonoids in the Rutaceae and especarlly the subfamily Aurantioideae is discussed. Two new coumarins, 7-isopentenyloxy-8-isopentenylcoumiain from *Choisya arizonica* and *C. mollis* and 2′,3′-dihydroxydihydrosuberosin from *Coleonema album* have been isolated and their structures determined.

LIMONOID bitter principles are C_{26} triterpenes whose occurrence is confined to the closely related plant families Rutaceae and Meliaceae.[1] The botanical distribution of the various rutaceous plants among the three major subfamilies correlates remarkably well with their limonoid content.[2] On this basis, the major subfamilies of the Rutaceae can be ranked in an order according to their increasing ability in effecting C-19 oxidation of limonoids, e.g. Toddalioideae, Aurantioideae and Rutoideae. Even though the Rutaceae has been a well investigated family[3] limonoids have been reported from only a relatively small number of genera. More extensive knowledge of the botanical distribution of limonoids would provide a sound basis for their use as chemotaxonomic indicators in this family. This paper reports recent results on the occurrence of limonoids in genera of the Rutaceae.

Choisya is a rutaceous genus native to North America.[4–6] Initial chemical work on the species *C. ternata* H.B.K., a widely cultivated ornamental shrub, resulted in the isolation of skimmianine (1) (Fig. 1), evoxine (2) and a new furoquinoline alkaloid, choisyine.[7] Subsequent work by Johns, *et al.*[8] established the structure of choisyine (3).

The closely related species, *C. arizonica* Standl. and *C. mollis* Standl., which grow in the canyons of mountain ranges of Southeastern Arizona, have now been shown to contain lupeol (9), limonin (11), the furoquinoline alkaloids, skimmianine (1), kokusaginine (4) and choisyine (3) as well as the coumarins xanthyletin (6) and 7-isopentenyloxy-8-isopentenylcoumarin (8) (Fig. 1). TLC also indicated the presence of the alkaloid evoxine (2). The

---

* Part XI in the series "Citrus Bitter Principles". For Part X see D. L. Dreyer, *Tetrahedron* **26**, 5745 (1970).

[1] For a review see, D. L. Dreyer, *Fort. Chem. Org. Naturstoffe* **26**, 190 (1968).

[2] D. L. Dreyer, *Phytochem.* **5**, 367 (1966).

[3] J. R. Price, in *Chemical Plant Taxonomy* (edited by T. Swain), Chap. 15, p. 429, Academic Press, London (1963).

[4] A. Engler, *Die Naturlichen Pflanzenfamilien* (edited by A. Engler and K. Prantl), 2nd Edn., Vol. 19a, p. 240. Englemann, Leipzig (1931).

[5] P. C. Standley, *Trees and Shrubs of Mexico*, Vol. 23, part 3, p. 537. Contributions from the United States National Herbarium, Smithsonian Institution, Washington, D.C. (1923).

[6] C. H. Muller, *Amer. Midland Nat.* **24**, 729 (1940).

[7] V. I. Frolova, A. I. Ban'kovskii and M. B. Volynskaya, *Med. Prom. SSSR* **12**, 35 (1958); *Chem. Abstr.* **53**, 11761 (1959); V. I. Frolova and A. B. Kuzovkov, *Zh. Obshch. Khim.* **33**, 121 (1963); *Chem. Abstr.* **59**, 683 (1963).

[8] S. R. Johns, J. A. Lamberton and A. A. Sioumis, *Austral. J. Chem.* **20**, 1975 (1967).

identity of lupeol (9) followed from its high resolution mass spectrum and similarity to published mass spectral data on lupane derivatives.[9]

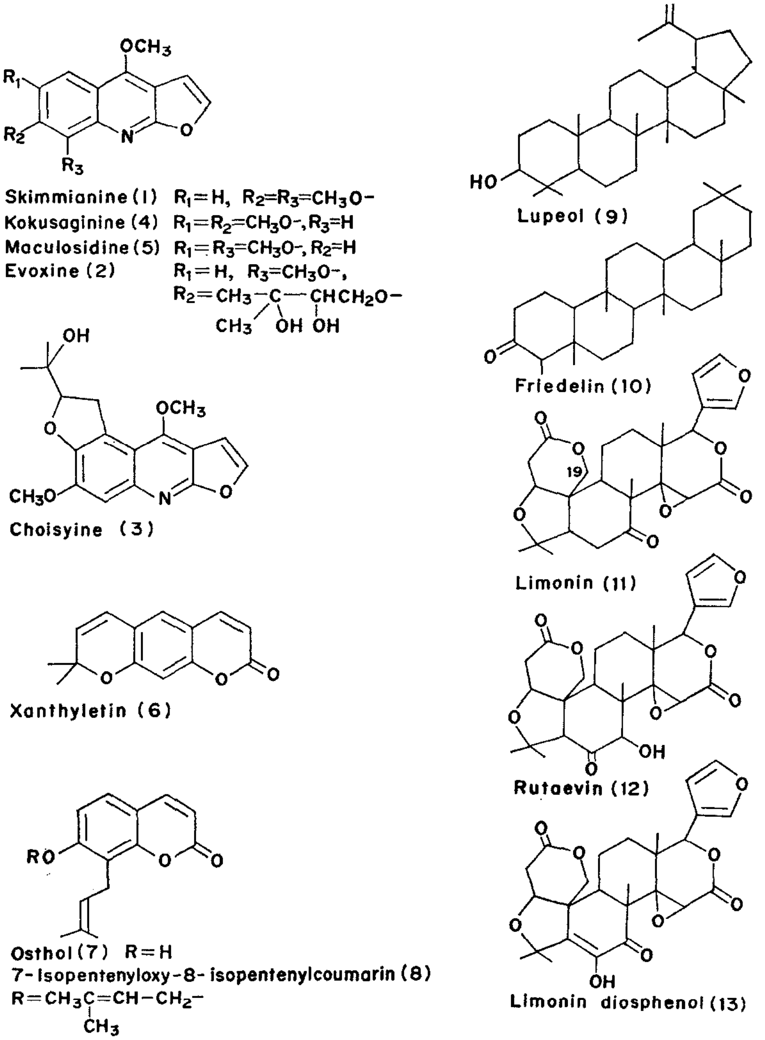


FIG. 1. EXTRACTIVES OF *Choisya* AND *Esenbeckia*.

7-Isopentenyloxy-8-isopentenylcoumarin (8), m.p. 66·5–66·8°, is blue fluorescing on TLC and its structure was established on the basis of spectroscopic evidence. High resolution mass spectrometry indicated the molecular formula $C_{19}H_{22}O_3$ and its UV spectrum was identical with that of osthol (7),[10] establishing the nature of the ring system. The NMR spectrum confirmed the osthol substitution pattern[11] and showed that the substituents were isopentenyl groups. The mass spectral fragmentation pattern was completely consistent with the proposed structure. A relatively weak molecular ion was consistent with the presence of

---

[9] H. BUDZIKIEWICZ, J. M. WILSON and C. DJERASSI, *J. Am. Chem. Soc.* **85**, 3688 (1963).

[10] W. L. STANDLEY, A. C. WAISS, JR., R. E. LUNDIN and S. H. VANNIER, *Tetrahedron* **21**, 89 (1965).

[11] See NMR spectrum of osthol, D. L. DREYER and A. LEE, *Phytochem.* **8**, 1499 (1969).

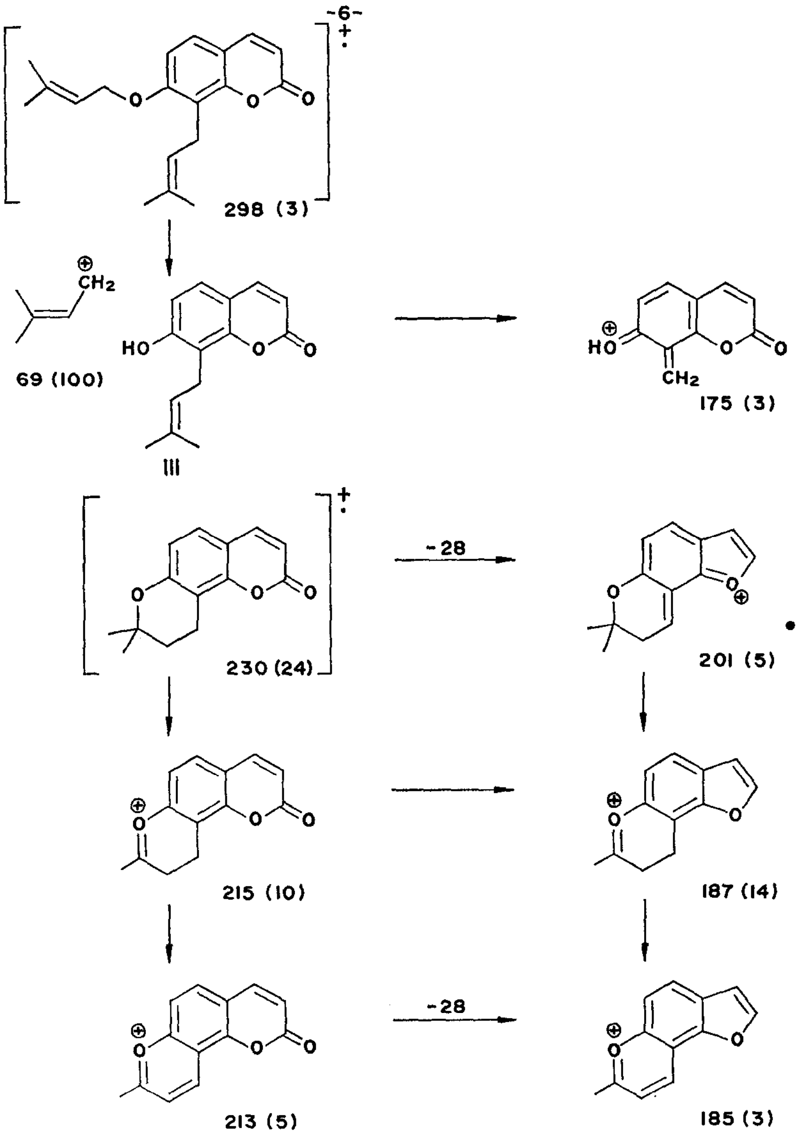


FIG. 2. STRUCTURAL ASSIGNMENTS FOR MAJOR MASS SPECTRAL FRAGMENTS OF COMPOUND 8.

an allyl ether group. Cleavage of the isopentenyl ether side chain gives a five carbon fragment, *m/e* 69, which was the base peak. Structures representing the major fragmentation peaks are outlined in Fig. 2. These data indicate structure 8 for the new coumarin.

The extracts of *C. arizonica* were indistinguishable in composition from those of *C. mollis* by TLC. The crude extracts of *C. arizonica* were compared with those of *C. ternata* by TLC. Spraying with Ehrlich's reagent[12] established the presence of limonin (11) in *C. ternata*. For the most part the extracts of *C. arizonica* and *C. ternata* were very similar and spots on TLC for the previously described evoxine (2), skimmianine (1) and choisyine (3) as well as many unidentified components were common to both species. Only limited amounts of *C. ternata*

---

[12] D. L. DREYER, *J. Org. Chem.* 30, 749 (1965).

extracts were available. It proved possible to isolate friedelin (10), skimmianine (1) and kokusaginine (4) from these extracts after chromatography on alumina. Pentacyclic triterpenes are not common in the Rutaceae, although lupeol (9) has been reported from the rutaceous species *Zanthoxylum clavaherculis* SW.,[13] *Z. pluviatile*[14] and *Medicosma cunninghamii.*[15] Friedelin (10) has also been reported to occur in *Citrus.*[16]

Some authorities[6] have suggested that the northernmost species of *Choisya* should be classified as a separate genus. The near identity of *C. arizonica* and *C. mollis* with *C. ternata* in chemical composition fails to support such a division of the genus.

Esenbeckia is a rutaceous genus of about 30 species, native to the tropical Americas.[17,18] *Esenbeckia hartmanii* H.B.K. is the northernmost species of this genus, occurring in the Mexican States of Sonora and Sinaloa.

Extracts of *E. hartmanii* yielded, after chromatography on alumina, limonin (11), rutaevin (12) and the furoquinoline alkaloids, skimmianine (1) and maculosidine (5). In addition, the presence of limonin diosphenol (13) was indicated by TLC spraying with Ehrlich's reagent.[12] The extracts also gave a positive Mg/HCl test for flavonoids, but no attempt was made to isolate these compounds. Skimmianine, flindersiamine, maculine and rutaevin have been isolated from *E. febrifuga* Juss.[19]

The fruit of *Coleonema album* (*Diosma album*) was also examined for limonoids by TLC with negative results. Extracts of the fruit yielded, after chromatography, a coumarin, m.p. 134–135·5°. Its UV spectrum resembled that of a 7-alkoxycoumarin. The IR spectrum showed hydroxy absorption as well as the usual coumarin carbonyl band at 1730 cm^{-1}. The NMR spectrum of the coumarin showed resonances for a methoxy group, two aromatic singlets and the usual coumarin AB doublet for H-3 and H-4. These data indicated a 6-alkyl-7-alkoxycoumarin system. Further NMR signals were present which were consistent with 2,3-dihydroxydihydrosuberosin (16) for the coumarin. This compound has been previously prepared by Rondest *et al.*[20] from the naturally occurring 7-hydroxy derivative a constituent of *Evodia belahe* Baillon (Rutaceae). In addition, they prepared compound 16 from suberosin via the oxide.

In a similar manner, in this study suberosin (14) was converted to its oxide (15) by peracid oxidation. The oxide (15) was hydrolyzed by dilute acid to the synthetic diol (16) which proved to be identical in all respects with the natural coumarin.

Fruit of *Pamburus missionis* (*Atalantia missionis*)[21] was also examined for limonoids. Evidence for the presence of limonoids was inconclusive with the limited amount of plant material available. Chromatography of the extracts yielded the same severine ester as previously isolated from *Severinia buxifolia* (Poir.) Ten. (Rutaceae).[22]

The extracts from whole fruit of *Eremocitrus glauca* have been re-examined[2] for limonoids.

---

[13] J. Tomko, A. T. Amad, J. L. Beal and R. W. Doskotch, *Lloydia* **30**, 231 (1967).

[14] J. E. T. Corrie, G. H. Green, E. Ritchie and W. C. Taylor, *Austral. J. Chem.* **23**, 133 (1970).

[15] E. Bianchi, C. C. J. Culvenor and J. A. Lamberton, *Austral. J. Chem.* **21**, 2357 (1968).

[16] A. Weismann, A. Meisels and Y. Mazur, *J. Org. Chem.* **20**, 1173 (1955).

[17] Ref. 5, p. 281.

[18] Ref. 6, p. 535.

[19] J. C. Vitagliano and J. Comin, *Anales Asoc. Quim. Argentina* **58**, 59 (1970); *ibid.*, submitted for publication.

[20] J. Rondest, B. C. Das, M. Ricroch, C. Kan-Fan, P. Potier and J. Polonsky, *Phytochem.* **7**, 1019 (1968).

[21] W. T. Swingle and P. C. Reece, *The Citrus Industry* (edited by W. Reuther, H. J. Webber and L. D. Batchelor), Vol. 1, p. 263, University of California Press, Rev. Edition (1967).

[22] D. L. Dreyer, *Tetrahedron* **23**, 4612 (1967).

Careful TLC and spraying with Ehrlich's reagent[12] gave clear evidence for the presence of limonin. In a similar manner, the presence of limonin in the fruit of *Hesperethusa crenulata* (Roxb) Roem. could be established. Seed extracts of *Triphasia trifolia* (Burm. f.) P. Wils. gave isopimpinellin. The presence of limonin (11) in *Triphasia* has been demonstrated by Maier *et al.*[23]

According to the Engler and Prantl system[4] the genera *Choisya* and *Esenbeckia* are both located in the subfamily Rutoideae. It has been previously pointed out[2] that only the more highly oxidized limonoids, limonin (11), rutaevin (12) and limonin diosphenol (13) are found in plants belonging to this subfamily. The isolation of limonin (11) and rutaevin (12) from *Choisya* and *Esenbeckia* is consistent with this limonoid distribution pattern. Similarly, the presence of only limonin (11) in the genera *Hesperethusa, Eremocitrus* and *Triphasia* is consistent with their placement in the subfamily Aurantioideae.

As noted in the introduction, the structures of limonoids show a remarkably good correlation between oxidation levels and their botanical distribution among the three main subfamilies of the Rutaceae. Thus, limonoids with an intact C-19 methyl group are found in the subfamily Toddalioideae. Minor amounts of these same limonoids, as well as major amounts of their C-19 oxidation product, limonin (11) itself, are found in plants of the subfamily Aurantioideae. Finally, plants of the subfamily Rutoideae are so efficient at C-19 oxidation that limonin precursors are not accumulated but only limonin (11) and its further oxidation products, rutaevin (12) and limonin diosphenol (13) are found. Hence, the three main subfamilies of the Rutaceae can be ranked according to their increasing ability to affect C-19 oxidation, e.g. Toddalioideae, Aurantioideae and Rutoideae. One might therefore, arrange the main subfamilies of the Rutaceae on an evolutionary ladder according to the oxidation level of their limonoids.

Current knowledge on the distribution of limonoids among the main subfamilies of the Rutaceae is summarized in Fig. 3. Further consideration of the limonoid distribution shows some promising correlations in the Aurantiodeae. The classification of this subfamily is displayed in Fig. 4.[24]

Those plants occurring in Tribe 1, the Clauseneae, apparently do not produce limonoids. Several studies on *Micromelum* species have been reported[25] and limonoids have not been observed. A search for limonoids, using Ehrlich's reagent as a selective spray reagent with thin layer chromatography,[2,26] among plants belonging to genera of subtribes 2 and 3, *Glycosmis*,[2] *Claisena*[27] *Murraya*,[28] and *Merrillia*[27] has failed to disclose the presence of limonoids. The limonoid pattern among plants of Tribe II, Citreae, is however, quite different. Plants from only two genera of subtribe 1, Triphasiinae, have been examined, e.g. *Triphasia* and *Luvunga*. Limonin has been found in *Luvunga eleutherundra* Dalz[29] and in *Triphasia trifolia* (Burm. f.) P. Wils.[23] Thus those species of this subfamily which have been investigated all contain limonoids.

The bulk of the genera occurring in subtribe 2 of the Citreae have been the subject of

---

[23] V. P. Maier, S. Hasegawa and E. Hera, Abstracts 9th Annual Meeting Phytochemical Society (1969)

[24] Ref. 22, p. 195.

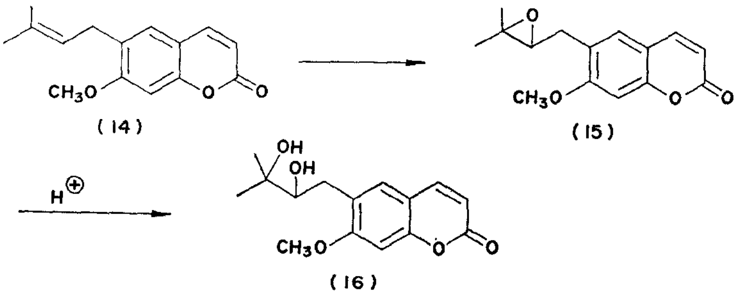
[25] J. A. Lamberton, J. R. Price and A. H. Redcliffe, *Austral. J. Chem.* **20,** 973(1967); A. Chatterjee, C. P. Dutta and S. Bhattacharyya, *Sci. and Cult.* **33,** 371 (1967); *ibid.* **34,** 366 (1968).

[26] D. L. Dreyer, *J. Org. Chem.* **30,** 749 (1965).

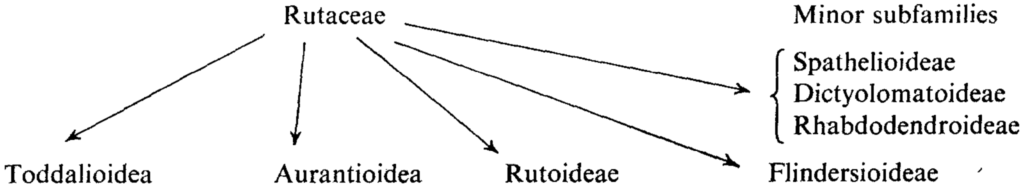
[27] D. L. Dreyer, unpublished results.

[28] D. L. Dreyer, *J. Org. Chem.* **33,** 3574 (1968).

[29] A. K. Ganguly, T. R. Govindachari, A. Manmade and P. A. Mohamed, *Ind. J. Chem.* **4,** 292 (1966).



chemical investigations, e.g. *Citrus*,[2] *Microcitrus*,[2] *Poncirus*,[2,26,30] *Eremocitrus*, *Fortunella*,[2] *Atalantia*,[31] *Hesperethusa* and *Severinia*[22] have been investigated chemically. All of these genera contain limonoids except *Severinia*. A determined effort to detect limonoids in *Severinia buxifolia* (Poir.) Ten. has, up to now, been unsuccessful.[22]



Toddalioidea	Aurantioidea	Rutoideae	Flindersioideae
Phellodendron (9–11)	*Triphasia* (3)	*Evodia* (120)	*Flindersia* (21)†
Helietta (6)	*Luvunga* (12)	*Choisya* (4)	
Casimiroa (5)	*Hesperethusa* (1)	*Dictamnus* (1)	
Vepris (2)	*Atalantia* (11)	*Calodendrum* (2)	
	Fortunella (4)	*Esenbeckia* (About 30)	
	Eremocitrus (1)		
	Poncirus (1)		
	Microcitrus (6)		
	Citrus (16)		
	Swinglea(1) †		

FIG. 3. DISTRIBUTION OF LIMONOIDS BY SUBFAMILIES AND GENERA IN THE RUTACEAE.*

* The number of species in each genus follows the generic name.

† *Flindersia* and *Swinglea* contain tetracyclic triterpenes which appear to be intermediates in limonoid biogenesis.

The search for limonoids has been extended to four genera of subtribe 3, e.g. *Swinglea*,* *Aegle*,[27,32] *Aeglopsis*,[33] and *Feronia*[34] with negative results. The available data thus suggests that limonoids are not present in plants of subtribe 3.

---

[30] T. TOMIMATSU, *Chem. and Pharm. Bull. Japan* **17**, 1723 (1969).

[31] M. R. THAKAR and B. K. SABATA, *Ind. J. Chem.* **7**, 870 (1969).

[32] Previous studies on *Aegle marmelos* Correa have also failed to report limonoids; A. CHATTERJEE and S. K. ROY, *Science and Culture* **23**, 1061 (1957); *Chem. Abstr.* **52**, 7338 (1958); A. CHATTERJEE and S. BOSE, *J. Ind. Chem. Soc.* **29**, 425 (1952); *Chem. Abstr.* **47**, 10544 (1953); R. N. CHAKRAVARTI and B. DASGUPTA, *J. Chem. Soc.* 1580 (1958); A. CHATTERJEE, S. BOSE and S. K. SRIMANY, *J. Org. Chem.* **24**, 687 (1959).

[33] D. L. DREYER, *J. Org. Chem.* **33**, 3658 (1968).

[34] This genus has not been investigated in the author's laboratory. However, limonoids have not been reported in several published studies of this genus; R. D. TIWARI and P. D. SATTSANGI, *Bull. Nat. Inst. India* **31**, 187 (1965); *Chem. Abstr.* **66**, 54662 (1967); J. P. TEWARI, *ibid.* **66**, 102471 (1967).

* See footnote * on p. 705.

Tribe I. Clauseneae

- Subtribe 1. Micromelinae
  - *Micromelum* (9)
- Subtribe 2. Clauseninae
  - *Glycosmis* (35)
  - *Clausena* (23)
  - *Murraya* (11)
- Subtribe 3. Merrilliinae
  - *Merrillia* (1)

Tribe II. Citreae

- Subtribe 1. Triphasiinae
  - *Wezelia* (9)
  - *Monanthocitrus* (1)
  - *Oxanthera* (4)
  - *Merope* (1)
  - *Triphasia* (3)*
  - *Pamburus* (1)
  - *Luvunga* (12)*
  - *Paramignya* (15)

Tribe II (cont.)

- Subtribe 2. Citrinae
  - *Severinia* (6)
  - *Plecospermium* (5)
  - *Burkillanthus* (1)
  - *Limnocitrus* (1)
  - *Hesperethusa* (1)*
  - *Citropsis* (11)
  - *Atalantia* (11)*
  - *Fortunella* (4)*
  - *Eremocitrus* (1)*
  - *Poncirus* (1)*
  - *Clymenia* (1)
  - *Microcitrus* (6)*
  - *Citrus* (16)*
- Subtribe 3. Balsamocitrinae
  - *Swinglea* (1)
  - *Aegle* (1)
  - *Afraegle* (4)
  - *Aeglopsis* (2)
  - *Balsamocitrus* (1)
  - *Feronia* (1)
  - *Feroniella* (3)

FIG. 4. CLASSIFICATION OF THE SUBFAMILY AURANTIOIDEAE.[24]

The number of species in each genus is given in parenthesis. Genera in which limonoids have been found are starred.

The above analysis depends upon accepting considerable negative evidence. This appears warranted upon considering two points; (1) the sensitivity of TLC with Ehrlichs' reagent as a method for detecting limonoids on a small scale in crude plant extracts and (2) the general ease of isolation of limonoids due to their ease of crystallisation. In any event, these correlations suggest that plants of the subtribe Triphasiinae would be a fruitful area for investigating new limonoids.

EXPERIMENTAL

NMR spectra were taken at 60 MHz and are given in δ relative to internal TMS. The relative areas of the peaks were consistent with their assignments. Coupling constants are given in Hz. TLC was run on silicic acid using a 1:1 ethyl acetate-chloroform solvent system.

Choisya *species. Choisya mollis* was collected on steep, rugged slopes in Sycamore Canyon, Atasco Mts., Santa Cruz Co., Arizona. *Choisya arizonica* was collected about 1 mile to the west of Middlemarch Pass on the N. slopes of Black Diamond Peak in the Dragoon Mts., N.E. of Tombstone, Arizona. Essentially identical results were obtained with each species.

The aerial portion of the plant material was dried, ground and extracted with acetone. Solvent was evaporated and the residue chromatographed on alumina. Hexane eluted lupeol (9), m.p. 196–198°, from EtOH. Major mass spectra peaks occurred at *m/e* (relative intensity) 69 (26), 81 (27), 95 (27), 109 (28), 121 (30), 135 (23), 189 (46), 191 (32), 203 (31), 207 (46), 218 (29), 411 (26), 426 (100). Found: M^+426·3820. $C_{30}H_{50}O$ requires M^+ 426·3861. The IR spectrum was identical with that of an authentic sample provided by Dr. J. A. Lamberton. Skimmianine (1), m.p. 175–177°, from EtOAc–hexane, was eluted with benzene. The IR spectrum was identical with that of a sample isolated from *Thamnosma montana.*[35] Limonin (11) was eluted with $CHCl_3$. The mother liquors from these operations were combined and extracted with 9% HCl. The acid extracts were neutralized and the recovered organic material was filtered through a short column of alumina to give choisyine, m.p. 186–187°, from acetone. Further crystallization of the choisyine mother liquors gave kokusaginine, m.p. 168–168·5° from EtOAc–hexane.

A further amount of ground plant material was soaked in hexane for several weeks. The hexane was decanted from the plant material, solvent evaporated from the extracts and the residue chromatographed on alumina. Benzene–hexane (9:1) eluted 7-isopentenyloxy-8-isopentenylcoumarin (8), m.p. 66·5–66·8°, from hexane–methylene chloride; λ_{max}^{EtOH} 258 (4000), 324 (10,900) nm; NMR δ7·39 (d, $J = 10$) H-4, 7·05 (d, $J = 8$) H-5, 6·60 (d, $J = 8$) H-6, 5·98 (d, $J = 10$) H-3, 5·8 (m) vinyl, 4·15 (d, $J = 6$) allyl ether methylene, 3·42 (d, $J = 8$) allyl methylene, 1·78, 1·63 vinyl methyls ppm (CCl_4). The mass spectrum showed major peaks at *m/e* (relative intensity) 69 (100), 175 (31), 185 (3), 187 (14), 201 (5), 215 (10), 230 (24), 298 (3); Found M^+ 298·1577; Calculated for $C_{19}H_{22}O_3$; M^+ 298·1569. Further elution with benzene gave fractions containing xanthyletin (6), m.p. 128·5–129·5°, from methylene chloride-hexane.

Foliage of *Choisya ternata*, collected at the Los Angeles County Arboretum, Arcadia, California, was dried and ground. The plant material was extracted with acetone. Solvent was removed from the extracts and the residue chromatographed on alumina to yield friedelin (10), identical in all respects with a sample isolated from cork wax. Skimmianine and kokusaginine were isolated and identified as described above for *C. mollis.* Limited amounts of plant material prevented a more detailed study of the constituents on a preparative scale. TLC and spraying with Ehrlich's reagent indicated the presence of limonin.

Esenbeckia Hartmanii *H.B.K.* Plant material was collected north of Guaymas, Mexico, highway 15 by km marker 1999 where a dry wash passes under the road. This is about 2 miles north of the turnoff of Bocachibampo Bay. The stems and branches were ground, dried and defatted about 2 hr with hexane. Subsequent to defatting the plant material was extracted 24–36 hr with benzene. During solvent removal, rutaevin (12) crystallized. Its IR spectrum was identical with that of a sample isolated from *Calodendrum capense.*[36] Chromatography of the residue on alumina, with $CHCl_3$ as eluent, provided limonin (11), m.p. 276–278° d; from CH_2Cl_2-*iso*PrOH. The IR spectrum was identical with that of an authentic sample. The mother liquors were combined and extracted with 9% HCl. The neutralized extracts were extracted with EtOAc. Solvent volume was reduced and after a small amount of hexane was added, a crop of skimmianine (1) was obtained. Recrystallized from acetone, skimmianine (1) showed m.p. 178–180°. Further workup of the mother liquors gave maculosidine (5), m.p. 183·5–185·5° from acetone. The IR spectrum was identical with that of a sample. provided by Dr. P. R. Jefferies. TLC of the mother liquors indicated the presence of limonin diosphenol (13).

Coleonema album. Dried and ground fruit of *Coleonema album*, collected at the University of California Botanical Garden, Berkeley, Calif., was extracted with acetone. Solvent was removed from the benzene extracts and the residue chromatographed on alumina. Fractions eluted with benzene were worked up to give 2′,3′-dihydroxydihydrosuberosin (16), m.p. 134–134·5°, from EtOAc–hexane; ν 1725 cm^{-1} (carbonyl); λ_{max}^{EtOH} 224, ~244, ~300, 332 nm; NMR δ7·56 (d, $J = 9$) H-4, 7·25(s) H-5, 6·74(s) H-8, 6·13 (d, $J = 9$) H-3, 3·87 methoxy, 3·14–2·34 (m) —$CH_2C\underline{H}$—O—, 1·32, 1·28 C-methyls ($CDCl_3$).

Suberosin oxide (15). To a solution of 100 mg of suberosin (14) in $CHCl_3$ was added 90 mg of *m*-chloroperbenzoic acid and the solution allowed to stand overnight at room temp. After workup, the oxide was recrystallized from hexane and benzene–hexane, m.p. 118–119°; yield 88%; ν 1710 cm^{-1} (carbonyl); λ_{max}^{EtOH} 224, ~245, ~300, 334 nm; NMR δ7·59 (d, $J = 9$) H-4, 7·27(s) H-5, 6·79(s) H-8, 6·23 (d, $J = 9$) H-3, 3·97(s) methoxy, 3·17–2·67 (m) —$CH_2C\underline{H}$—O—, 1·41, 1·37 C-methyls. (Found: C, 69·0; H, 6·29. $C_{15}H_{16}O_4$ required C, 69·21; H, 6·20%).

DL-2′,3′-*Dihydroxydihydrosuberosin* (16). The oxide (15) was converted to the diol (16) in 89% yield by refluxing with 5% aq. oxalic acid for 30 min; m.p. 133–134°. It was identical by IR, m.p. and R_f on TLC with natural (16). (Found: C, 64·7; H, 6·52. $C_{15}H_{18}O_5$ required: C, 64·8; H, 6·56%).

Pamburus missionis. Dried and ground fruit of *Pamburus missionis*, obtained from the U.S. Plant Introduction Station, Coral Gables, Florida, was extracted with benzene. TLC gave no evidence for limonoids. Solvent was removed from the extracts and the residue chromatographed on alumina. Fractions eluted with hexane–benzene deposited severine ester on standing. After recrystallization from EtOAc–hexane it was identical in all respects with a sample isolated from *Severinia buxifolia.*

Triphasia trifolia. Seeds of *T. trifolia* were dried, ground and extracted with acetone. Solvent was removed from the extracts and the residue filtered through a short column of alumina. Workup of the filtrates gave isopimpinellin, m.p. 140–143°, from EtOAc–hexane. The IR, UV and NMR spectra were identical with those of authentic samples.[19]

Extracts of *Eremocitrus glauca* and *Hesperethusa crenulata* fruit, obtained from the Citrus Research Center, University of California, Riverside, were examined for limonoids by previously described[2] TLC methods.

[35] D. L. Dreyer, *Tetrahedron* **22**, 2923 (1966).

[36] D. L. Dreyer, *J. Org. Chem.* **32**, 3442 (1967).

Acknowledgements—The authors are indebted to Prof. P. R. Jefferies for the sample of maculosidine, to Dr. W. C. Taylor for the sample of suberosin, to Dr. J. A. Lamberton for the sample of lupeol, to W. Anderson, H. Smith and M. Kei for the mass spectra and to Dr. E. McClintock and Austin Griffiths, Jr. for identification of plant material.

Key Word Index—Rutaceae; Chemotoxonomy; limonoids; coumarins.