FLAVONOLS, LEUCO-ANTHOCYANINS, CINNAMIC ACIDS, AND ALKALOIDS IN DRIED LEAVES OF SOME ASIATIC AND MALESIAN SIMAROUBACEAE

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SUMMARY

Herbarium specimens of 13 species of the *Simaroubaceae* were investigated on phenolic compounds present in their hydrolised leaf extracts and on the presence of alkaloids (table 2). Leucoanthocyanins, myricetin, gallic acid, ellagic acid, as well as alkaloids were demonstrated to occur rather frequently in this family.

The relationships between the *Simaroubaceae* and *Rutaceae* and the position of the genera *Irvingia* and *Suriana* are briefly discussed: the *Simaroubaceae* and *Rutaceae* seem to be closely related not only morphologically but also biochemically.

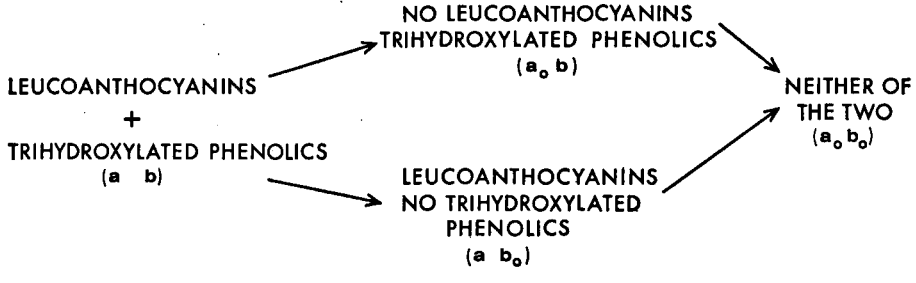
Irvingia seems to fit rather well in the *Simaroubaceae* (except for the assumed lack of bitter principles).

Suriana deviates much more from all other species investigated. This stimulates further research to check the recent proposal of Gutzwiller concerning the classification of this genus.

INTRODUCTION

The occurrence and distribution of distinct types of phenolic compounds (Bate-Smith 1962) and of alkaloids (Hegnauer 1958, 1963) may offer valuable additional characters to plant taxonomy.

According to Bate-Smith leucoanthocyanins occur predominantly in woody plants, especially those belonging to families generally assumed to be relatively primitive. The same holds true for phenolic compounds containing three vicinal hydroxylic groups (gallic acid, ellagic acid, leucodelphinidin, and myricetin). Taxa assumed to represent a higher evolutionary level (e.g. many families of the *Sympetalae*) as a rule do not contain leucoanthocyanins and these trihydroxylated phenols (except sinapic acid and delphinidin in flowers). Evolution of patterns of phenolic compounds in angiosperm leaves is supposed by Bate-Smith to have proceeded as follows:



If this scheme holds good and irreversibility of evolution is accepted it is clear that taxa containing leucoanthocyanins and trihydroxylated phenolic compounds cannot be derived from taxa lacking such constituents.

Besides having an assumed bearing on problems of phylogeny the patterns of phenolics of leaves may be a valuable taxonomic character. The same holds true for alkaloids. Often whole genera or families are characterised by presence or absence of these constituents. Furthermore distinct types of alkaloids are often characteristic at generic or family level.

As far as was known trihydroxylated phenolic compounds (gallic acid, ellagic acid) but no leucoanthocyanins occur in the *Simaroubaceae*. Moreover, the family is chemically characterised by the occurrence of quassiin and related bitter principles; the latter, however, seem to be absent in *Irvingia*. Several species contain quinones in the wood and in rare instances alkaloids were reported to be present in simaroubaceous plants.

The *Rutaceae* are chemically mainly characterised by the occurrence of essential oils, many types of coumarins, and alkaloids, some of which seem to be highly characteristic for this family. Flavonoids and biogenetically related phenolic compounds often occur in high concentration. Leucoanthocyanins and trihydroxylated phenolic compounds (myricetin, leucodelphinidin, but not ellagic acid and gallic acid) were observed by Bate-Smith (1962) to be present in members of the subfamily *Rutoideae* but not in members of the subfamilies *Toddalioideae* and *Aurantioideae*. Another chemical character of the *Rutaceae* can be found in the limonoid bitter principles which occur in all three subfamilies mentioned but are by no means ubiquitous in them. In the first instance the *Rutaceae* and *Simaroubaceae* appear to be biochemically rather remote:

group of compounds	*Simaroubaceae*	*Rutaceae*
gallic and ellagic acids	+	not known
leucoanthocyanins	not known	occurring frequently
bitter principles	C_{26}	C_{20} and C_{19}
benzoquinones	+	not known
coumarins	not known	ubiquitous
alkaloids	as far as known rarely occurring	ubiquitous
essential oils	traces only	large amounts

This summary needs some comment, however. The bitter principles of both families seem to be intimately related biogenetically. Furthermore, the *Rutaceae* are phytochemically much more intensively explored than the *Simaroubaceae*. For this reason the summary provides clearly no adequate reflection of the chemical relation between the two families.

Taxonomically the *Simaroubaceae* are closely related to the *Rutaceae*. This relation is even so close that Engler (1874) said: 'Wir sind genötigt alle diejenigen Formen aus der Reihe der Geraniales, welche sich äusserlich an eine der verschiedenen Rutaceen-Gruppen anschliessen, in ihrem anatomischen Verhalten aber in der angegebenen Weise von denselben sich unterscheiden, zu den Simaroubaceae zu rechnen.'

The present investigation intends to get more information about the phenolic constituents of the leaves of members of the *Simaroubaceae* and of the frequency of the occurrence of alkaloids in this family. Furthermore, I hoped to find additional characters for distinguishing the genera and species I revised in the Flora Malesiana (Nooteboom 1962).

MATERIALS AND METHODS

The present investigation is concerned with leaves only. With the exception of *Ailanthus altissima* only dried leaves from herbarium specimens were available.

Alkaloids: 500 mg of dried leaves were macerated for 24 hours with 5 ml of 2 % hydrochloric acid. The clear extracts were tested for alkaloids with the reagents of Bouchardat, Mayer, and Dragendorff. Alkaloids were presumed to be present if all three reagents produced a precipitate which dissolved after addition of ethanol.

Phenolic constituents: According to the directions of Bate-Smith (1954, 1962) hydrolised leaf extracts were prepared. The phenolics were extracted from these extracts with ether and thereafter with isopentanol. This results generally in less complex chromatographic patterns than extraction with isopentanol alone and accordingly makes the interpretation of the chromatograms more easy. In each instance four solvents were used for paper chromatography of the ether and isopentanol extracts.

a) 6 % acetic acid;
b) toluene/acetic acid/water (4/1/5), used as indicated by Bate-Smith;
c) butanol/acetic acid/water (6/2/1);
d) Forestal solvent (acetic acid/HCl/water 30/3/10).

Chromatograms were first examined in day light and in u. v. light before and after fuming with ammonia vapor. Furthermore, some confirmatory sprays were used. These were:

1) basic lead acetate for flavonols (kaempferol becomes yellow, quercetin yellow-orange, myricetin orange in day light);
2) 2 % ferric chloride in methanol (caffeic acid becomes greyish-green, ellagic acid blue green, gallic acid greyish blue);
3) Hoepfners reagent (sodium nitrite 2 g, acetic acid 2½ g, methanol 100 ml): this gives a red-brown colour with caffeic acid which is not changed after spraying with 0,5 N methanolic potassium hydroxide; ellagic acid gives a yellow-brown colour changing to orange.

R_f values and colour reactions were compared with those of authentic compounds. The characteristics found to be most useful for identification are summarised in table 1.

RESULTS

The species investigated and the compounds tentatively identified with a reasonable degree of certainty are summarized in table 2.

Ferulic acid, sinapic acid, and p-coumaric acid could never be demonstrated to be present with certainty. P-coumaric acid may be present in the following species: *Ailanthus altissima*, *A. triphysa*, *Eurycoma apiculata*, *E. longifolia*, *Harrisonia brownii*, *H. perforata*, *Picrasma javanica*, *Quassia indica*, and *Soulamea amara*, according to my observations.

Leaves of herbarium specimens produce rather complex chromatographic patterns and the identification of the cinnamic acids is rather difficult. Bate-Smith (1962) got different results with *Ailanthus altissima*, *A. giraldii* Dode, and *A. vilmoriana* Dode regarding caffeic acid and p-coumaric acid. I consider these taxa, of which the last one was described from a cultivated tree, as conspecific.

When more than one species of a genus was investigated, the chromatographic patterns generally resembled each other (table 2). Leaves of *Eurycoma longifolia* and *E. apiculata*, for instance, produced identical chromatographical patterns. On the other hand some

Table 1: R_f-values and colour tests of some phenolic constituents of leaves.

Compound	R_f - values				U.V.	U.V. + ammonia	Lead-acetate	$FeCl_3$	Hoepfner	
	a	b	c	d					without KOH	with KOH
K.	0	0	0,90	0,56	± yellow	intens.	yellow			
Q.	0	0	0,68	0,40	± yellow	intens.	yellow-orange			
M.	0	0	0,50	0,30	± yellow	intens.	orange			
F.	0,31	0,32	0,85	0	blue	bright blue		orange	light yellow	yellow brown
Caff.	0,28	0	0,80		blue	bright blue		greyish-green	red-brown	red-brown
S.	0,28	0,16	0,77		blue	greenish		rose	yellow brown	brown
p-C.	0,40	0,06	0,90		—	purple		yellow	light yellow	light yellow
Gent.	0,58	0	0,85		purple-blue (bright)	id. to greenish		—	—	—
E.	0,04	0	0,30	0,30	soft blue	± pale yellow		blue-green	yellow brown	orange
G.	0,40	0	0,60	0,60	—	—		greyish-blue	dirty yellow	yellow brown
Cy.	0	0		0,50	red	purple				
D.	0	0		0,30	red	purple				

K. = kaempferol; Q. = quercetin; M. = myricetin; F. = ferulic acid; Caff. = caffeic acid; S. = sinapic acid; p-C. = p-coumaric acid; Gent. = gentisic acid; E. = ellagic acid; G. = gallic acid; Cy. = cyanidin; D. = delphinidin.

differences were observed between two specimens of *Harrisonia perforata*. This may be due to the fact that herbarium specimens were available only. The conditions during the preparation of herbarium specimens, of course, affect the patterns of phenolics in leaves, especially in the tropics, where different drying techniques are used. Other factors which may influence the observable composition of leaf extracts are condition and time of keeping. Further factors which may affect the concentration (and hence detectability) of distinct phenolic compounds in leaves are season of collection and the age and environment of the plant.

Nothwithstanding these restrictions it is the experience that well kept herbarium specimens are generally sufficient to get information about general biochemical trends of taxa [compare also Rheede van Oudtshoorn (1963), Bate-Smith (1965)].

DISCUSSION

My observations demonstrate that the *Simaroubaceae* are chemically more closely allied to the *Rutaceae*, especially to the subfamily *Rutoideae*, than hitherto supposed. In the *Rutoideae* Bate-Smith (1962) recorded the occurrence of myricetin, leucodelphinidin, quercetin, leucocyanidin, kaempferol, and caffeic acid. Nothwithstanding this, he placed the *Rutaceae* in his group (a b_0) (see p. 309). However, according to data derived from literature at least the *Rutoideae* would better fit in his group (a b). The *Simaroubaceae* can also be included in this group.

Table 2: Phenolic constituents and alkaloids in dry leaves of herbarium specimens of *Simaroubaceae*

Species [1]	Compounds identified [2]									Remarks [3]
	Alkaloids	K.	Q.	M.	Caff.	E.	G.	Cy.	D.	
a. Ailanthus altissima (Mill.) Swingle . . .	+	?	+	—	++	—	+++	—	—	Caff. +
b. Ailanthus triphysa (Dennst.) Alst. . . .	—	—	+	?	+	++	+++	+	—	LA_1 +, LA_2 +
c. id.	—	—	+	?	+	++	+++	+	—	LA_1 +, LA_2 +
d. Brucea javanica Bl. .	+	—	—	—	?	—	—	—	—	
e. Eurycoma longifolia Jack	—	—	+	—	?	—	—	+	+	LA_1 +, LA_2 +
f. Eurycoma apiculata Benn.	—	—	+	—	?	—	—	+	+	LA_1 +, LA_2 +
g. Harrisonia brownii A. Juss.	+	—	+	+	?	+	+	+	—	
h. Harrisonia perforata (Blanco) Merr. . . .	+	—	+	+	?	+	+	+	—	
j. id.	+	—	—	—	?	+	+	—	—	
k. Irvingia malayana (Oliv.) *ex* Benn. . .	+	—	—	+	+	+	++	—	—	E+
l. Picrasma javanica Bl.	+	+	+	+	?	+	+	—	—	
m. Quassia indica (Gaertn.) Nooteboom	+	—	—	+	—	+	—	—	—	
n. Soulamea amara Lamk	+	—	—	—	?	—	—	—	—	
o. Soulamea soulameoides (A. Gray) Nooteboom	×	×	×	×	×	×	×	×	×	LA_2 +
p. Suriana maritima L. .	—	—	—	—	—	—	—	—	—	

+ present; — not detectable; × test not performed.

1) Material from the Rijksherbarium, Leyden (except a.).

a: *L.E.P. 1377*, 4-9-1961, cult. in hort. Pharm. Lab., Leyden, deposited in the herbarium of the Lab. voor Exp. Plantensyst., Leyden. b: *van Borssum Waalkes 564*, Java, Pulau Penaitan, 17-9-1951 (sublim.), c: *P.N.H. 37913*, Mindoro, 12-4-1958. d: *de Raadt 42*, S. Sumatra, 16-5-1948. e: *Jacobs 5195*, Sarawak. 20-8-1958 (sublim.). f: *C.F. 33867*, Mal. Pen., Kepong, 24-2-1934. g: *Pleyte 36*, Tanimbar Is, 7-8-1956 (sublim.). h: *San A 176*, N. Borneo, Sandakan, 22-5-1951 (sublim.). j: *P.N.H. 39486*, Luzon, 27-6-1958 (sublim.). k: *Soekaria 81*, S. Sumatra, 14-9-1953 (sublim.). l: *KK & SS 245*, Bali, 1-7-1958 (sublim.). m: *N.G.F. 4605*, New Guinea, Morobe Distr., 29-9-1952 (sublim.). n: *Anderson 803*, Caroline Is, 6-12-1949. o: *Smith 5664*, Fiji. p: *Taylor 461056*, Marshal Is, Bikini Atoll, 25-3-1946.

2) For abbreviations see table 1. See for LA_1 and LA_2 note 3.

3) The following test-tube tests (Bate-Smith 1954) for confirmation of ellagic acid, caffeic acid, sinapic acid, and leucoanthocyanidins were performed: ½ g dry leaf is bruised for one minute with 10 ml of methanol containing 10 drops of 30 % acetic acid. The filtered extract is shaken with petroleum ether to remove most of the chlorophyll. To one half of the only slightly green extract 10 drops of 2 % sodium nitrite is added. Colour change to brown and appearance of a dirty brown precipitate indicate much ellagic acid (E^+). Next 10 % sodium carbonate is added until the mixture is slightly alcaline: an intense red colour changing to light brown by addition of some drops of 4N sodium hydroxide indicates sinapic acid (S^+) and an orange brown colour becoming deep red after the addition of sodium hydroxide indicates caffeic acid (Caff.+). The other half of the filtrate is shaken with some coal and filtered. Next 10 drops of concentrated hydrochloric acid are added, and some drops of a saturated ethanolic solution of vanillin; a red colour indicates leucoanthocyanins and/or catechins (LA_1^+). A second test for leucoanthocyanins is performed as follows: ½ g dry leaf was extracted at room temperature with 5 ml 2N hydrochloric acid for half an hour; if the clear extract turns red after heating this indicates the presence of leucoanthocyanidins (LA_2^+).

Alkaloids were demonstrated by me to occur as frequently in the *Simaroubaceae* as they do in the *Rutaceae*. In the latter family alkaloids formally derived from anthranilic acid occur most frequently. Besides these compounds benzyltetrahydroisoquinoline alkaloids are present in some genera. Several of the isoquinolines found in the *Rutaceae* are widespread in the *Polycarpicae* (magnoflorine and berberine) and others are characteristic for *Papaveraceae* (allocryptopine and chelerythrine) (Hegnauer 1963).

It is obvious that more research concerning the chemical nature of the alkaloids of the *Simaroubaceae* has to be done before we are sufficiently well informed about the biochemical relations of this family with other families.

Flavonoid compounds seem to occur frequently in the *Simaroubaceae* except in *Suriana*. Besides the flavonols myricetin, quercetin, and kaempferol other flavonoid compounds were indicated by several unidentified yellow spots on most of the chromatograms (except in *Suriana*).

It seems that the occurrence of ellagic acid and gallic acid, both common in the *Simaroubaceae*, can be considered as a character distinguishing this family from most others generally accepted as being closely related *(Rutaceae, Burseraceae, Meliaceae)*. It must be kept in mind, however, that in future, as work proceeds, these compounds possibly will be found in the above mentioned families. In one family of the *Geraniales* of Engler, the *Geraniaceae*, both compounds occur frequently.[1]

Morphologically, the *Simaroubaceae* and the *Rutaceae* are related to each other by the following characters: disk in both families intrastaminal, annular or cushion shaped, or gynophorous; androecium usually obdiplostemonous, stamens in the *Simaroubaceae* often, in the *Rutaceae* seldom with an adaxial scale at the base; in both families the epipetalous stamens sometimes wanting or reduced to staminodes; carpels free or only connate by the styles, or more rarely entirely connate; ovules anatropous, hanging, with adaxial raphe and upwards pointing micropyle, 2 or 1 in the *Rutaceae*, usually only 1 in the *Simaroubaceae*; embryo large, straight or curved, seeds in the *Rutaceae* with or without endosperm, in the *Simaroubaceae* endosperm absent or scant. In fact the only constant difference between these families is the presence of oilcontaining cavities in the *Rutaceae*, their absence in the *Simaroubaceae*. But in *Harrisonia perforata* (exactly?) similar pellucid spots occur in the margins of the leaves of some specimens (Forman 1958, Nooteboom 1962).

The observations reported in this paper indicate a similar close biochemical relationship between the *Rutaceae* and *Simaroubaceae*.

The genus *Irvingia* which, together with the other genera of the subfamily *Irvingioideae*, is often referred to a separate family *(Irvingiaceae)*, fits well in the scheme of the *Simaroubaceae*, not only by the structure of the flower, but also by the occurrence of similar chemical compounds (table 2).

The same does not hold for the genus *Suriana*. The thin coloured petals, the suprabasal style, the 2 basal ovules with downwards pointing micropyle, the isobilateral leaves with anisocytic stomata are all characters differentiating *Suriana* from other *Simaroubaceae*. The chromatograms of *Suriana maritima* were characterised by few spots, none of which could be identified and none of which corresponded to spots observed on chromatograms of other *Simaroubaceae*. All these facts tend to favour the proposition of Gutzwiller (1961) who excluded *Suriana* from the *Simaroubaceae*. She considered the monotypic family

[1]) In passing it may be remarked that a morphological character linking the *Simaroubaceae* with the *Geraniaceae* is found in the simaroubaceous genus *Kirkia* Oliv. possessing a schizocarp in which after splitting the 1-seeded mericarps hang from a central columella.

Surianaceae to be rather more closely related to the *Sapindaceae* and *Chrysobalanaceae*. In my opinion it would be worthwile to check Gutzwiller's suggestion by extensive anatomical and phytochemical investigations. The striking deviation of the chromatograms of *Suriana maritima* L. observed by me may provisionally be interpreted as providing additional evidence for the isolated position of *Suriana* within the *Simaroubaceae*.

It is not yet possible to answer the question whether chromatographic patterns of hydrolised leaf extracts offer additional characters for the distinction of species and genera in the *Simaroubaceae* because too few representatives have been examined phytochemically. The data in table 2 show that further research along this line may prove to be profitable, especially with regard to the occurrence of myricetin, leucoanthocyanins, and gallic and ellagic acid.

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