International Organization for Chemical Sciences in Development

Working Group on Plant Chemistry

CHEMISTRY, BIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF AFRICAN MEDICINAL PLANTS

Proceedings of the first International IOCD-Symposium
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Edited by

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African traditional healer and *Harpagophytum procumbens* (Pedaliaceae)
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6. Chemistry and biological properties of the African Combretaceae

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Introduction

Of the many genera that comprise the African Combretaceae, the two largest, *Combretum* and *Terminalia*, occur in most parts of Africa where they are often the dominant groups as regards numbers. They consist of climbers, shrubs and trees and are readily characterised by fruits with wing-shaped appendages.

Although traditional healers throughout Africa have used species of the Combretaceae for the treatment of a wide range of disorders, only about twenty five out of the approximately ninety nine African species of *Combretum* have been subjected to any form of scientific study; with the exception of a few species of the *Terminalia, Anogeissus* and *Guiera*, virtually nothing has been reported on the phytochemistry of any of the remaining genera. This family thus represents a practically unexplored reservoir of potentially useful substances.

Metabolites isolated so far include alkaloids (*G. senagalensis*), tannins (*A. schimperi*), flavonoids (*C. micranthum*), and amino acids (*C. zeyheri*); substituted phenanthrenes from various heartwoods; a rich variety of triterpenoid acids and their saponins mainly of the cycloartane (*C. molle*) and oleane (*C. imberbe*) types; and a series of unique stilbenes, their glucosides, and macrocyclic lactones called combretastatins (*C. caffrum, C. kraussii*). Many of the *Combretum* species exude gums that are similar in composition and properties to gum arabic.

Electron microscope and chemical investigations have shown that the acidic triterpenoid mixtures isolated from the *Combretum* are secreted onto the surface of the leaves and the fruit through epidermal, scale-like trichomes. The anatomy of the trichomes and the mixtures of triterpenoid acids and saponins that they secrete are both species specific, which raises interesting questions and provides an important combination of taxonomically useful characters for this genus.

Certain of the metabolites show cytotoxic, molluscicidal, anti-HIV, anti-microbial and anti-inflammatory activity, and several of the triterpenoid acid

mixtures have strong inhibitory effects on plant germination and seedling growth. Isolation of the toxins that render the fruit of the *Combretum* poisonous to animals and cause violent and prolonged hiccuping in humans (hence the name "hiccup nut" given to many species) has not yet been accomplished.

Taxonomic classification of the Family

The Combretaceae family belongs to the order Myrtales (Fig. 6.1) which is divided into two sub-families of which only the Combretoideae is of interest. Of the two tribes comprising this sub-family, only the Combreteae, which is further divided into three sub-tribes, is of relevance in Africa. The genera from these sub-tribes that are African and have been investigated are *Combretum, Terminalia, Anogeissus,* and *Guiera*; the number of species for each genera is given in

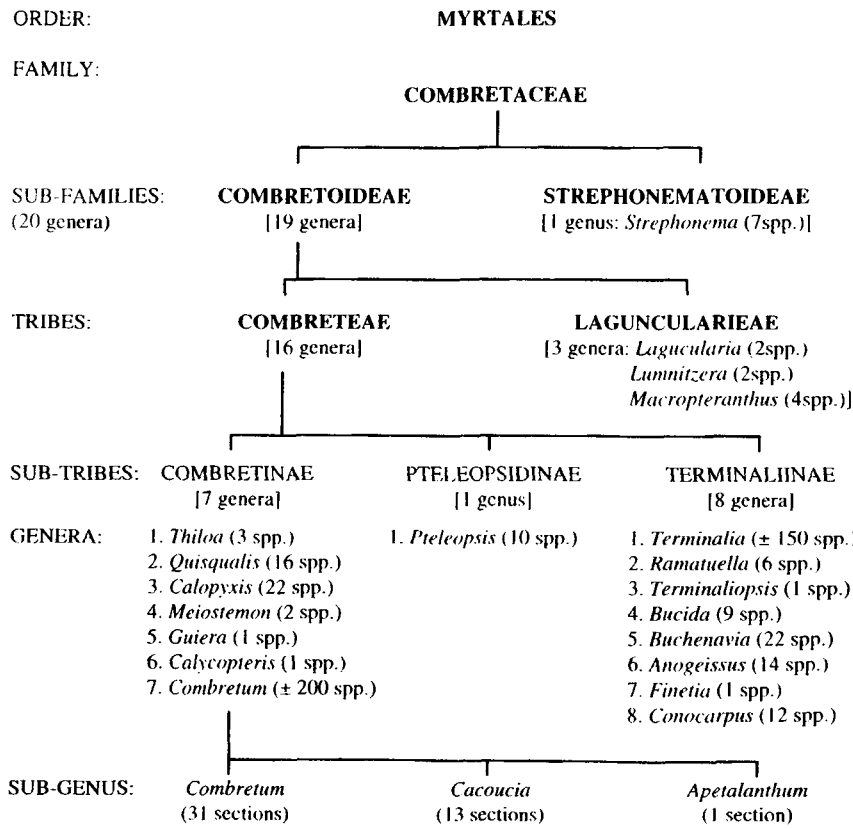


Fig. 6.1. Taxonomic classification of the family Combretaceae.

parenthesis in Fig. 6.1. Of these the genus *Combretum*, which contains about one hundred African species in two sub-genera *Combretum* and *Cacoucia* (*Apatalanthum* is an American genus), has been investigated the most extensively and is the most interesting. Consequently any investigation of the properties of the African Combretaceae is essentially an investigation of the genus *Combretum*.

Use of the Combretaceae in Traditional Medicine

Reports in the literature (Watt *et al.* 1962; Gelfand *et al.* 1985; Kokwaro 1976) indicate that traditional healers throughout Africa have confined themselves almost exclusively to the use of species from the genus *Combretum* and to a lesser extent the *Terminalia* in the treatment of a wide range of maladies (Table 6.1). Although the use of the leaves and bark from *Combretum* species is widespread, the winged fruits, which are produced in great abundance, are never used in medicine (nor are they eaten by wild animals) because of their reported toxicity to humans.

The interest in the use of members of the Combretaceae in traditional medicine has led to a burgeoning interest in this family with an increasing number of species now under investigation for biological activity.

Pharmacological activity in the Combretaceae

Despite the wide use of species of this family by traditional healers, very little of pharmacological importance had been reported until recently. The first scientific study carried out was that on the west-African drug "Kinkeliba" made from the leaves of *C. micranthum* (Paris 1942). This drug used in French Sudan, Senegal and Nigeria for the treatment of biliary fever, colic and vomiting, has a cholagog and diuretic action and is antimicrobial (gram positive and gram negative). In the last two decades a series of stilbenes and dihydrostilbenes (the combretastatins) with potent cytotoxic activity and acidic triterpenoids and their glycosides with molluscicidal, antifungal, antimicrobial and antiinflammatory activity have been isolated from species of *Combretum* (Rogers 1989). These will be dealt with in more detail later.

More recently lignans with HIV-1 reverse transcriptase inhibitory activity have been isolated from *A. acuminata* (Rimondo *et al.* 1994) and 3,4,5-tri-O-galloylquinnic acid, also shown to have anti-HIV activity, has been isolated from *G. senegalensis* (Mahmood 1993). Antimicrobial activity found in *Anogeissus* species in the Sudan has been attributed to 3,3',4'-tri-O-methylflavellagic acid extracted from the bark (Almagboul 1988), and chewing sticks from *A. leiocarpa* are reported to prevent dental caries (Sanui 1983). Leaf decoction extracts from *C. glutinosum* from Senegal strongly inhibit hepatitis B virus antigen (HBsAg) *in vitro* and angiotensin-converting enzyme (Poussel *et al.* 1993).

Table 6.1 : Some Medicinal uses of the Combretaceae

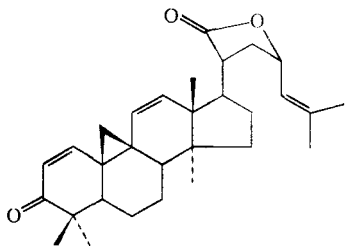
COMBRETUM		
C. apiculatum	E. Africa:	Snake bite, scorpion bite, bloody diarrhoea, leprosy.
	S. Africa:	Abdominal disorders, conjunctivitis.
	Zimbabwe:	Weak body
C. erythrophyllum	S. Africa:	Fattening tonic for dogs.
	Zimbabwe:	To reduce size of vaginal orifice.
C. fragrans	E. Africa:	Chest coughs, syphilis.
	Zimbabwe:	Aphrodisiac.
C. glutinosum	Senegal:	Hepatic disease, antihypertensive, diuretic, bronchial disease.
C. hereroense	E. Africa:	Bilharzias.
	Zimbabwe:	Headache, infertility in women.
C. imberbe	S. Africa:	Coughs, colds
	Zimbabwe:	Diarrhoea, to drive away bad spirits bilharziasis.
C. microphyllum	Zambia:	Lunacy.
	Zimbabwe:	Lucky charm.
C. molle	E. Africa:	Hookworm, stomach ache, snakebite, leprosy, fever, dysentery, chest complaints, anthelmintic.
	Zambia:	Headaches.
	Malawi:	Anthelmintic, snake bite.
	Zimbabwe:	Abdominal pains, diarrhoea, headaches, convulsions, infertility in women, to stop bleeding after childbirth, to fatten babies, as a dressing for wounds.
C. platypetalum	Zambia:	Swelling caused by mumps.
	Zimbabwe:	Pneumonia, abdominal pains, diarrhoea, antiemetic, dysmenorrhoea, infertility in women, earache, epistaxis, haemoptysis.
C. zeyheri	E. Africa:	Toothache, cough
	Tanzania:	Scorpion bite, diarrhoea with blood.
	Zambia:	To arrest menstrual flow, eye lotion, embrocation, diarrhoea.
	Zimbabwe:	Diarrhoea with blood, abdominal disorders.
TERMINALIA		
T. brachystemma	Zimbabwe:	Haematuria, bilious vomiting, constipation, diarrhoea.
T. sericea	E. Africa:	Bilharziasis, stomach troubles.
	Botswana:	To arrest purging.
	S. Africa:	Stomach disorders, bilharziasis, diabetes.
	Tanzania:	Stomach disorders, bilharziasis.
	Zimbabwe:	Diarrhoea, epistaxis, prolapsed rectum of infants, backache, to widen vagina, wounds, abdominal pains, worms on arms, antiemetic, infertility in women, tonic, depressed fontanelle, sore throat, gonorrhoea, bilharziasis, abortion, dilated birth canal.
T. stenostachya	Zimbabwe:	Epilepsy, antidote for poison.
T. brevipes	Somalia:	Hepatitis, malaria.

Toxicity of the Combretum

The "nut" inside the four winged fruit or samara from species such as *C. collinum* and *C. zeyheri* is comparable in size and appearance to a pecan nut yet, despite being produced in vast amounts, it is not consumed by wild animals or the indigenous people. On a continent where starvation was endemic, this is a clear indication that the fruits are toxic, although there is only one case on record where this has been clinically proven (BSA Police 1970).

In an attempt to identify the toxins in the *Combretum* fruit, cytotoxic studies on the fruit of fifteen species where carried out using brine shrimp and other assays (Panzini *et al.* 1993). Compounds isolated included a series of acidic triterpenoids and their glycosides, non-proteinogen aminoacids and several combretastatins and their glucosides-these compounds will be discussed fully later. Although showing other forms of activity, none of these compounds appeared responsible for the toxicity.

More recently it has been reported that five women have died in Zimbabwe after inserting material from *C. erythrophyllum* into their vaginas to reduce the size (Mavi, S., National Herbarium, Harare, Zimbabwe, personal communication, 1996). More unreported deaths have possibly occurred, since this is a widespread practice in rural areas of Zimbabwe. Compounds thus far isolated from *C. erythrophyllum* include a series of unusual cycloartane dienone lactones (**1-3**) from the leaves (Rogers, C.B. University of Durban-Westville, unpublished data) and combretastatin glucosides from the roots (Brookes, B., Mangosuthu Technicon, Durban, personal communication, 1996)



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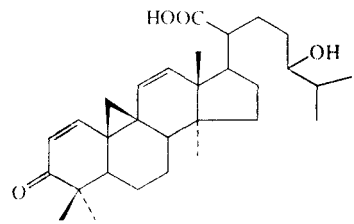

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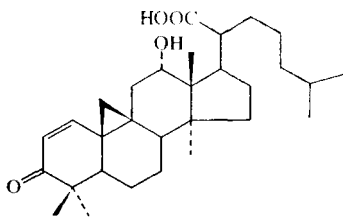


(3)

Metabolites isolated from the Combreaceae

The isolation of metabolites can be divided roughly into five significant stages starting in chronological order with the investigation of the extractives from *C. micranthum* in 1942 (Paris 1942) followed by the isolation of the substituted phenanthrenes (Letcher and Nhamo 1971); the gum exudates (Anderson and Bell. 1974); the acidic triterpenoid glycosides (Rogers *et al.* 1976) and the stilbenes and dihydrostilbenes (Pettit *et al.* 1988a).

Aminoacids and other nitrogen compounds

The activity of the drug "Kinkaleba" from the leaves of *C. micranthum* has been attributed to simple nitrogen compounds such as choline, betaine and combretins A and B (Paris 1942; Bassene 1989), whereas the presence of the amino acids *N*-methyl-L-tyrosine and its 4'-O-β-D-glucoside, 3-aminomethyl-L-phenylalanine, and 3-(3'-hydroxymethyl-L-phenylalanine) and its 3'-O-β-D-glucoside isolated from the fruit of *C. zeyheri* (Mwauluka *et al.* 1975a, 1975b; Panzini *et al.* 1993; Perosa 1992) has been attributed to fungal intrusion. With the exception of the simple indole alkaloids harman and eleagnine isolated from the roots of *G. senegalensis* (Combier 1977), there have been no other reports of alkaloids from the Combretaceae.

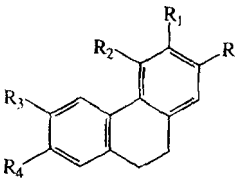
Substituted phenanthrenes and dihydrophenanthrenes

An investigation of the borer and termite resistance of *C.apiculatum* heartwood resulted in the isolation of seventeen substituted phenanthrenes and 9,10-dihydrophenanthrenes (**4-20**) from this tree and the heartwood of *C. molle, C. psidioides, C. hereroense* (Letcher *et al.* 1971, 1972 and 1973; Malan and Swinny 1993) and *C. caffrum* (Pettit *et al.* 1982). Certain of these compounds (**6, 17**) totally inhibited the growth of *Penicillium expansum* in antifungal tests (Malan and Swinny 1993) and three of the 9,10-dihydrophenanthrenes (**16, 18, 20**) isolated from *C. caffrum* showed reasonable antileukemic activity in the P-388 murine system (Pettit *et al.* 1988b).

Combretaceae gum exudates

Impetus for the study of these gums was supplied by the extreme Sahelian droughts of 1972-74, which devastated the *Acacia* population that supplied the bulk of the world's gum arabic requirements. The drought did not affect the *Combretum* forests further south and since Combretaceae gums are used extensively as adhesives and in other technological applications (*Anogeissus*

latifolia is the main component of gum ghatti), they were investigated as possible replacements for the *Acacia* gums (Anderson and Bell 1977).



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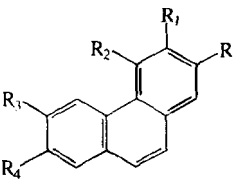


(12)-(20)

	R_1	R_2	R_3	R_4	R_5	PLANT
4	OCH_3	OCH_3	OH	OH	OH	*C. apiculatum*
5	OH	OCH_3	OCH_3	OH	OCH_3	*C. apiculatum*
6	OCH_3	OCH_3	OH	OCH_3	OH	*C. molle*, *C. apiculatum*
7	OCH_3	OCH_3	OCH_3	OCH_3	OH	*C. caffrum*
8	OH	OH	OCH_3	OCH_3	OH	*C. apiculatum*
9	OCH_3	OH	OCH_3	OH	OH	*C. apiculatum*
10	OCH_3	OH	OCH_3	OCH_3	OH	*C. apiculatum*
11	OCH_3	OH	OCH_3	OCH_3	OCH_3	*C. apiculatum*
12	OCH_3	H	OH	OCH_3	OH	*C. apiculatum*
13	OCH_3	OCH_3	OH	OCH_3	OH	*C. apiculatum*, *C. molle*
14	OH	OCH_3	OCH_3	OH	OCH_3	*C. apiculatum*, *C. molle*
15	OCH_3	OCH_3	OH	OH	OH	*C. molle*, *C. apiculatum*
16	OCH_3	OCH_3	OCH_3	OH	OH	*C. caffrum*, *C. apiculatum*, *C. hereroense*
17	OH	OCH_3	OCH_3	OCH_3	OCH_3	*C. caffrum*
18	OH	OCH_3	OCH_3	OCH_3	OH	*C. caffrum*, *C. apiculatum*, *C. psidioides*
19	OCH_3	OCH_3	OCH_3	OCH_3	OH	*C. apiculatum*, *C. psidioides* *C. caffrum*
20	OCH_3	OH	OCH_3	OCH_3	OCH_3	*C. apiculatum*

Gum exudates from eleven *Combretum,* two *Terminalia* and two *Anogeissus* species have been examined and classified as arabinogalactan proteins (Anderson and Bell 1974 and 1977; Anderson *et al.* 1990 and 1991) and "gum *Combretum*" has been characterised as shown in Table 6.2.

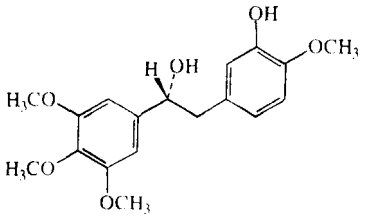
Because of their high acidity and tannin content,"*Combretum* gums" are not permitted in foodstuffs, although they have been found to be the most frequent adulterant of commercial gum arabic (Anderson *et al.* 1991). The overall similarity of the gums makes this deception difficult to detect.

Table 6.2. Characteristics of "Gum *Combretum*"

- Dextrorotatory
- Low in Nitrogen; high in rhamnose
- High methoxy content
- High zinc content
- High in aspartic acid and glycine, low in hydroxyproline
- High in tannin
- Markedly hygroscopic - tend to "block" in storage
- High molecular mass, high acidity

Stilbenes and dihydrostilbenes (Combretastatins)

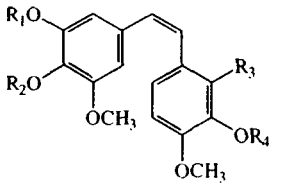
In 1979 an NIC screening programme found extracts from the Cape bushwillow, *C. caffrum*, to be cytotoxic and combretastatin (**21**) [*R*(-)-1-(3,4,5-trimethoxyphenyl)-2-(3-hydroxy-4-methoxyphenyl) ethanol], the first of a series of unique stilbenes highly active against the murine P-388 lymphocytic leukemia cell line, was isolated from the methylene chloride-methanol extract of the whole plant (Pettit *et al.* 1982).



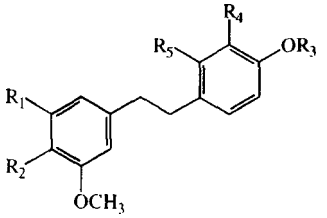
Combretastatin (**21**)

Large scale extraction of *C. caffrum* stemwood yielded further combretastatins designated A (**22-28**), B (**29-46**), C (**47**) and D (**48, 49**) according to the skeleton type (Pettit *et al.* 1987; Pettit and Singh 1987) plus a series of bibenzyls (Pettit *et al.* 1988c; Malan and Swinny 1993). In addition the fruit of *C. kraussii* yielded

combretastatins A-1 (**22**) and B-1 (**29**) and their corresponding 2'-O-β-D-glucosides (**28,44**) in considerable yields compared to the yields from *C. caffrum* (Pelizzoni *et al.* 1993). For reasons that are probably connected to recent uncharacteristic annual climatic variations in South Africa, attempts to isolate the glucosides from fruit produced in the last few seasons has proved unsuccessful!

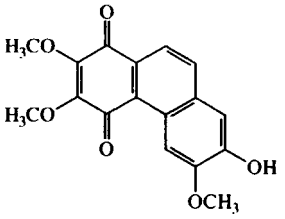


	R_1	R_2	R_3	R_4		PLANT
22	CH_3	CH_3	OH	H	combretastatin A-1	*C. caffrum*, *C. kraussii*
23	-CH_2-		OH	H	combretastatin A-2	*C. caffrum*
24	H	CH_3	H	OH	combretastatin A-3	*C. caffrum*
25	CH_3	CH_3	H	OH	combretastatin A-4	*C. caffrum*
26	H	CH_3	H	CH_3	combretastatin A-5	*C. caffrum*
27	H	CH_3	H	CH_3	combretastatin A-6 = (*E*)-combretastatin A-5	*C. caffrum*
28	CH_3	CH_3	OH	OGlc		*C. kraussii*

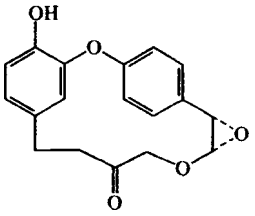


	R_1	R_2	R_3	R_4	R_5		PLANT
29	OCH_3	OCH_3	CH_3	OH	OH	combretastatin B-1	*C. caffrum*, *C. kraussi*
30	O-CH_2-O		CH_3	OH	OH	combretastatin B-2	*C. caffrum*
31	OCH_3	OCH_3	H	OH	H	combretastatin B-3	*C. caffrum*
32	OCH_3	H	H	OH	H	combretastatin B-4	*C. caffrum*
33	OCH_3	H	CH_3	OH	H		*C. caffrum*

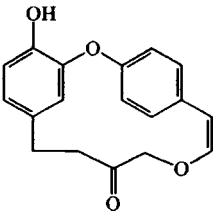
34	OCH_3	H	H	H	H		*C. caffrum*
35	OCH_3	OCH_3	H	H	H		*C. psidioides*, *C. caffrum*
36	OH	H	H	H			*C. apiculatum*
37	OH	H	CH_3	OH	H		*C. apiculatum*
38	OH	OCH_3	H	OCH_3	H		*C. apiculatum*
39	OH	OH	CH_3	OCH_3	H		*C. apiculatum*
40	OH	OCH_3	H	OH	H		*C. apiculatum*
41	OH	OH	CH_3	H	H		*C. apiculatum*
42	OH	OCH_3	H	H	H		*C. apiculatum*, *C. molle*
43	OCH_3	OH	H	H	H		*C. apiculatum*, *C. psidioides*
44	OCH_3	OCH_3	CH_3	OH	O-Glc		*C. kraussii*
45	OCH_3	OH	CH_3	OH	OH	combretastatin B-5	*C. kraussii*
46	OCH_3	OH	CH_3	OH	O-Glc		*C. kraussii*



combretastatin C-1 (**47**)



combretastatin D-1 (**48**)



combretastatin D-2 (**49**)

Whereas all the combretastatins show some cytotoxic activity, certain of these compounds are especially effective. In particular, combretastatins A-1 (**22**) and B-1 (**29**) are potent inhibitors of microtubule assembly *in vitro* and were, at the time, among the most potent inhibitors of the binding of colchicine to tubulin (Pettit *et al.* 1987). In addition, in tests on mammalian sensory neurons, combretastatin B-1 (**29**) had rapid and completely reversible effects on a variety of

potassium ion channels without any marked effects on calcium or sodium channels (Verotta *et al.* 1994).

However, the most potent cancer cell growth inhibitor of the series was found to be combretastatin A-4 (**25**) (Pettit *et al.* 1995). It is the most potent inhibitor of colchicine binding to tubulin yet discovered and inhibits tubulin polymerisation, retards strongly the growth of the murine lymphocytic leukemia L1210 and P388 cell lines as well as human colon cancer lines and is a potent antimitotic agent. As a result of this activity, combretastatins A-4 (**25**) and B-1 (**29**) are the subjects of patents (Pettit and Singh 1991, US Patent and Pelizzoni *et al.* 1994, IT Patent).

Bibenzyls and combretastatins have been found in trace quantities in fruit extracts of *C. bracteosum* and *C. zeyheri* (Lanfossi, M., University of Milan, unpublished data, 1993) and in *C. erythrophyllum* root bark (Brookes, B., Mangosuthu Technicon, Durban, personal communication, 1995).

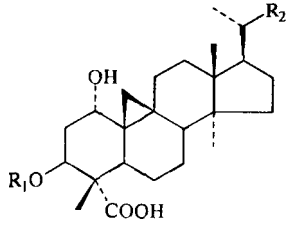
Acidic triterpenoids and their glycosides

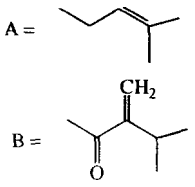
The serendipitous discovery that the triterpenoid molluscicide mollic acid (**50**) and its glycosides (**51-53**) are secreted as a surface coating on *C. molle* leaves and fruit through glandular, scale-like trichomes present on the leaf and fruit epidermis, has resulted in the isolation of a rich variety of acidic triterpenoids and their glycosides. Since these trichomes appear in great profusion on the epidermis of leaves and fruit of all species belonging to the subgenus *Combretum*, all other species of this subgenus were tested and found to have similar secretions. Both the composition of the mixture of triterpenoid acids and their saponins that are secreted and the anatomy of the trichomes responsible for the secretions have been found to be species specific. Thus trichome anatomy and trichome secretions provide an important combination of taxonomically useful characters for this subgenus and will be discussed later.

Since nearly all the acidic triterpenoids produce water soluble salts, the harvesting of the triterpenoids and their glycosides is achieved by simply washing fresh leaves with warm, 1% bicarbonate solution and acidifying the resultant solution (Lawton *et al.* 1991). Subjected to TLC analysis using a relatively polar solvent (ethyl acetate:chloroform:formic acid; 5:4:1) and the spray reagent *p*-anisaldehyde/c.H_2SO_4 in ethanol (5:5:90), each species gives a fingerprint of different coloured spots that is a powerful chemotaxonomic tool. In nearly all species studied so far, geographic and seasonal variation have little or no effect on the composition of the extracts (Carr and Rogers 1987).

Thus far the triterpenoids isolated from the *Combretum* belong almost exclusively to two distinct groups: *viz* 30-carboxy-1α-hydroxycycloartanes (**1-3, 50-56**) and 29-carboxy-1α-hydroxyoleanes (**57-69**) (Pegel and Rogers 1976, 1985; Rogers and Subramony 1988; Panzini *et al.* 1993; Rogers 1988, 1989a, 1898b; Osborne and Pegel 1985; Jossang *et al.* 1996). With the exception of the *C. molle* fruit extract, which contains both oleane and cycloartane triterpenoids and their mono- and bidesmosides, trichome secretions contain either one group or the other

(Panzini *et al.* 1993). There also appears to be a correlation between the size and morphology of the trichomes and group that is secreted. This will be discussed further-see Fig. 6.6.



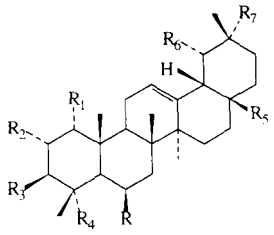


	R_1	R_2		PLANT
50	H	A	mollic acid	*C. molle*, *C. leprosum*
51	-D-glucosyl	A	molic acid glucoside	*C. molle*
52	-D-xylosyl	A	mollic acid xyloside	*C. molle*
53	-L-arabinosyl	A	mollic acid arabinoside	*C.molle*, *C. edwardsii*
54	H	B	jessic acid and its methyl ester	*C. elaeagnoides*
55	-L-arabinosyl	B	jessic acid arabinoside	*C. elaeagnoides*
56	-D-xyloside	B	jessic acid xyloside	*C. molle* (fruit)

Molluscicidal activity of the triterpenoids

The sodium salts of mollic acid glucoside (**51**), toxic to *Biomphalaria glabrata* snails at a concentration of 12 ppm (Rogers 1989), is the major 30-carboxy-1α-hydroxycycloartane glycoside trichome secretion washed from the leaf surface of *C. molle* by a hot, 1% $NaHCO_3$ solution (yield ± 0.8% dried and ± 0.25% fresh whole leaf). This salt qualifies as an ideal, economically viable, plant-derived molluscicide for use in Third World environs in Africa according to World Health Organisation criteria (Table 6.3).

Of the other triterpenoids tested, the sodium salt of imberbic acid (**57**) is toxic to *B. glabrata* snails at concentrations of 20 ppm whereas its bidesmosides (**58**) and (**60**) are inactive (Hostettmann, K., University of Lausanne, personal communication, 1987)



	R	R_1	R_2	R_3	R_4	R_5	R_6	R_7		PLANT
57	H	OH	H	OH	CH_3	CH_3	H	COOH	imberbic acid	*C. imberbe*
58	H	OH	H	OH	CH_2-O-^{4}AcRha	CH_3	H	COOH		*C. imberbe*, *C. padoides*
59	H	OH	H	O-Rha	CH_2-O-4AcRha	CH_3	H	COOH		*C. imberbe*, *C. padoides*
60	H	OH	H	O-Rha	CH_2O-Rha	CH_3	H	COOH		*C. padoides*
61	H	OH	H	O-Rha	CH_2O-Rha	CH_3	H	CH_2OH		*C. padoides*
62	H	OH	H	O-Rha	CH_2O-^{4}AcRha	CH_3	H	CH_2OH		*C. padoides*
63	H	OH	H	OH	CH_2O-Rha	CH_3	H	COOH		*C. imberbe*
64	H	OAc	H	OH	CH_2O-Rha	CH_3	H	COOH		*C. imberbe*
65	H	H	OH	OH	CH_2OH	COOH	OH	CH_3	arjungenin	*C. nigricans*
66	H	H	OH	OH	CH_2OH	COOGlc	OH	CH_3	arjunglucoside I	*C. molle* *C. nigricans*
67	OH	H	OH	OH	CH_3	COOH	OH	CH_3	arjunetin	*C. molle*
68	OH	H	OH	OH	CH_2OH	COOH	OH	CH_3	combregenin	*C. nigricans*
69	OH	H	OH	OH	CH_2OH	COOGlc	OH	CH_3	combre-glucoside	*C. nigricans*

Table 6.3. Advantages of mollic acid glucoside (**51**) as a molluscicide in the control of schistosomiasis in Africa

- *C. molle* is the most widespread *Combretum* in Africa occurring from the Transkei in the south and as far north as Arabia.
- A medium sized tree, it produces a high mass of leaves each season.
- The leaves are a renewable resource and are easier to harvest than berries or roots.
- The extraction process is cheap; a 200 litre drum, firewood and inexpensive $NaHCO_3$ are needed to prepare solutions.
- Unsophisticated labour would be able to carry out the process successfully.
- Mollic acid glucose is stable and has a low toxicity to mammals.

Allelopathic activity of epidermal leaf secretions from *Combretum* species.

It was observed that, although planted in thick coastal forest with ample rainfall, the area under the leaf canopy of a thicket bushwillow, *C. padoides*, remained barren. Since this tree and most other *Combretum* are deciduous, the area at the base of the tree is covered with a thick layer of leaves every autumn. To check whether the triterpenoid secretions on the leaves were responsible for this inhibition of undergrowth, 1% bicarbonate extracts from *C. padoides, C. molle, C. moggii, C. apiculatum, C. zeyheri, C. imberbe, C. celestroides, C. erythrophyllum* and *C. oxystachyum* were tested against a variety of commercially available seeds and seeds of *C. molle* (Rogers, 1991). Whereas seeds germinated readily in a 1% bicarbonate solution, germination was totally inhibited in almost ALL the solutions from the *Combretum*; the few seeds that did germinate did not grow. Furthermore, germination of *C. molle* seeds was significantly retarded by its own leaf extract although a solution of mollic acid glucoside (**51**), the main constituent of this leaf extract, did not have the same drastic inhibitory effects. As a control, a similar extract from *Albizia adianthifolia*, a local tree, enhanced germination and stimulated growth of the seedlings.

Intercontinental Chemotaxonomic links between African and South American *Combretum*

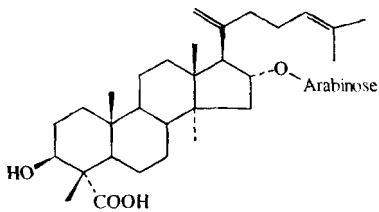
The isolation from the South American species, *C. leprosum*, of the cycloartenoid mollic acid (**50**) previously found only in several African *Combretum* species (Facundo 1993) establishes a direct "intercontinental" chemotaxonomic link between species on the two continents and presents intriguing phytochemical and

phytogeographic questions related to the ancestry and development of this genus (Table 6.4).

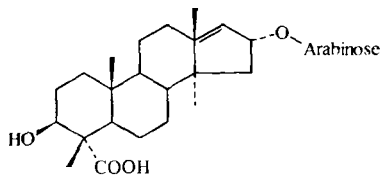
Table 6.4. "Intercontinental" *Combretum* relationships

	Approximate Number of Species			
SUBGENUS	Africa	Madagascar	Asia	S.America
Combretum	98	1	18	28
Cacoucia	60	5	8	5

Treatment of leaves from South American and Indian species with 1% bicarbonate solution has yielded mixtures of acidic triterpenoids similar to those found in South African species (Rogers, C.B., University of Durban-Westville, unpublished data) and the extract from the Brazilian liana, *C. rotundifolium*, yielded two acidic dammarane arabinofuranosides, (**70, 71**) (Rogers 1995). This shows that the epidermal trichomes function in the same way on all three continents.



(70)



(71)

These results give some idea of the age of the genus and its origin. Since the continents had separated by a significant amount approximately 120 million years ago and the separation was complete 64 million years later (Fig. 6.2), the *Combretum* must have been part of the flora of Gondwanaland ± 200 million years ago. The distribution of species in Table 6.3 supports the evidence that Africa formed the centre of Gondwanaland.

Fig. 6.2. Gondwanaland [----- Present range of the *Combretum*].

The anatomy of the epidermal trichomes and their mode of action in the *Combretum*

The discovery by electron microscopy that the often copious, sticky secretions present on young *Combretum* leaves were produced by glandular, scale-like trichomes that crowd the epidermis of all species of the subgenus *Combretum* (Fig. 6.3) (Stace 1969), coincided with the discovery that the secretions consisted of acidic triterpenoids and their glycosides (Lawton and Rogers 1991). Each trichome consists of a basal cell embedded in the epidermis and a two celled stalk subtending a cap (Fig. 6.4); it is the species specificity of the morphology of the cells in this one-cell-thick cap that has established this as such a valuable taxonomic character (Stace 1981).

Fig. 6.3. Surface of the leaf of *Combretum caffrum* showing trichomes in various stages of development.

In young trichomes, secretions accumulate within thewalls of the cap cells until the outer layer of the wall and cuticle break away (Fig. 6.5.) releasing the triterpenoid secretion as a sticky layer on the surface of the leaf. Evidence thus far suggests that there is a correlation between the trichome morphology and the class of triterpenoid secreted. Trichomes that are larger and have a complicated arrangement of cap cells secrete the oleane group of triterpenoids, whereas trichomes with smaller caps and less complicated cell patterns produce triterpenoids of the cycloartane group. This bifurcation in triterpenoid synthesis is illustrated in Fig. 6.6.

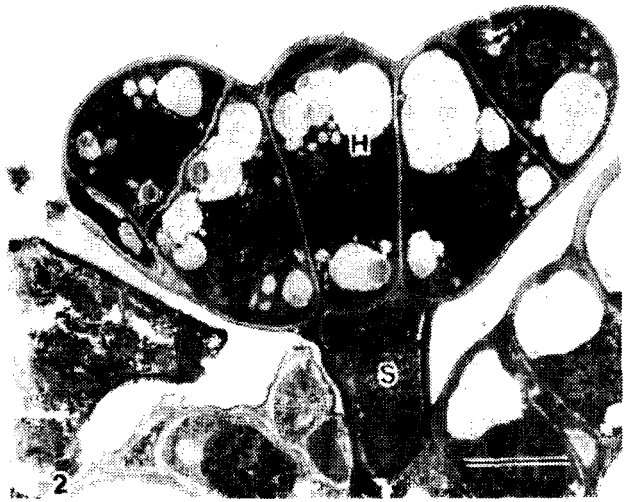


Fig. 6.4. Tangential longitudinal section through a trichome on a young leaf of *C. molle* showing a 2 celled stalk (S) subtending a head/cap (H). (Bar = 0.5mm).

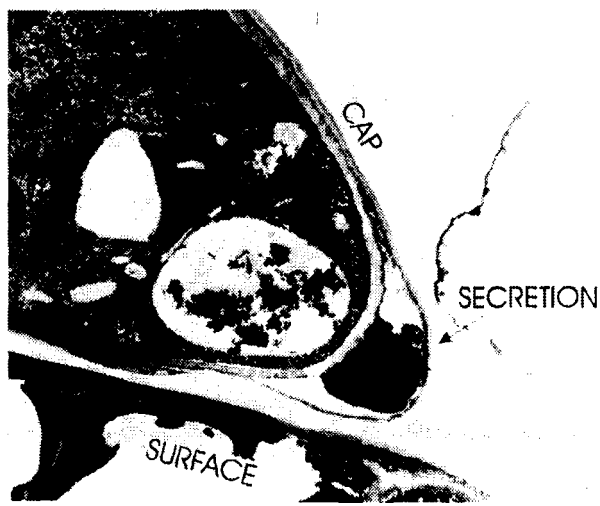


Fig. 6.5. Section through a trichome showing the outer layers of the wall and the cuticle about to rupture.

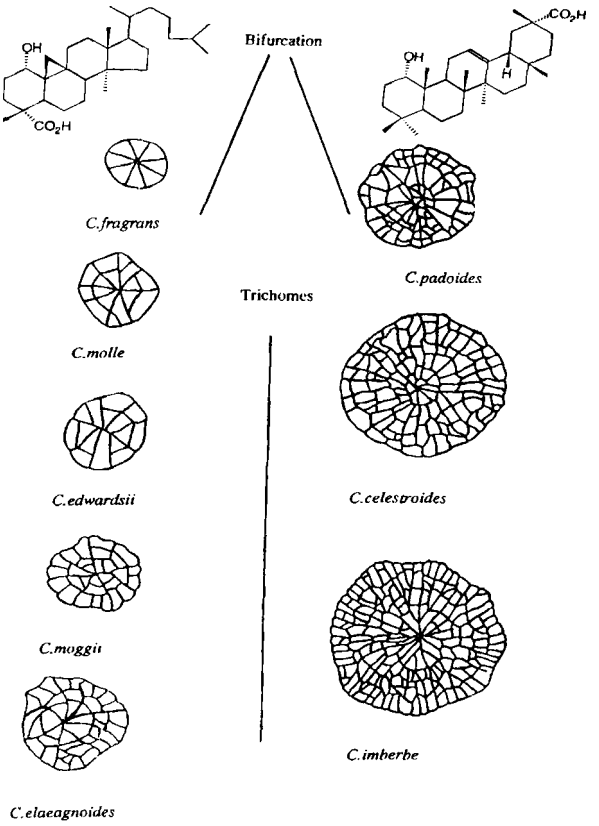


Fig. 6.6. Correlation of trichome anatomy with triterpenoid group secreted.

Since the composition of the acidic triterpenoid mixtures is also species specific, such relationships are to be expected. In particular, the triterpenoid mixtures and scale anatomy of *C. edwardsii, C. collinum, C. molle* and *C. moggii* are almost identical; the latter two species are so similar that this chemical evidence has convinced some botanists to review the distinction (Hennessy, E.F., University of Natal, personal communication, 1990).

Of less importance chemotaxonomically and of little taxonomic importance are the epidermal stalked glands that cover the epidermis of *Combretum* subgenus *cacoucia* species. The triterpenoid mixtures are much simpler and the stalked glands have no distinctive features.

Conclusion

The reported uses of the Combretaceae in traditional medicine has lead to a burgeoning interest in this family with an increasing number of genera other than the *Combretum* now under investigation. The isolation and activity of the combretastatins represents a significant advance in medicinal plant chemistry and the high yields and variety of still to be evaluated acidic triterpenoids from the leaf epidermis of the *Combretum* show promise as molluscicidal and allelopathic agents. The acidic triterpenoid "fingerprint" given by each *Combretum* species is now well established as a chemotaxonomic tool in the biology of this genus (yields are so high and the extraction procedure so simple, that *Combretum* leaves could prove a commercial source of these triterpenoids). Recent work shows that *Terminalia* species give similar "fingerprints" (Rogers, C.B., University of Durban-Westville, unpublished data).

Investigations of the genera *Anogeissus* and *Guiera* have yielded promising metabolites and initial investigations of the *Terminalia* (Rogers, C.B., University of Durban-Westville, unpublished data) are encouraging. No studies on the underground parts of any of the genera have been reported but, given the richness of the leaf and fruit extracts, these should provide a wealth of interesting metabolites.

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