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VOLATILE RESIN EXUDATE FROM STEM BARK OF *COMMIPHORA ROSTRATA*: POTENTIAL ROLE IN PLANT DEFENCE*

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IN MEMORY OF TONY SWAIN, 1922–1987

Key Word Index—*Commiphora rostrata*; Burseraceae; volatile resin; 2-alkanones; 2-alkanols; 3-alkanones; alkanals; 2,2-dimethylalkanols; anti-fungal activity; plant defence.

Abstract—The volatile resin exuded from the stem bark of *Commiphora rostrata* has been examined and 22 oxygenated alkane components identified by GC and mass spectrometry. The potential value of this material in the defence of the plant against predators and fungal pathogens is discussed.

INTRODUCTION

Commiphora rostrata is one of numerous species of the genus found in the arid area of northern Kenya, Somalia and southern Ethiopia [2]. Many *Commiphora* species produce oleo-gum-resins a number of which, notably myrrh, are of commercial significance [3]. *Commiphora rostrata* is a small deciduous tree rarely attaining a height of more than 3 m [4, 5]. The simple leaves are edible and are reported to have a flavour of oxalic acid [4]. The red-brown bark is smooth and contains copious amounts of a clear pungent resin which appears to be retained under pressure. When branches or twigs are cut or bent the resin is released both as a fine spray and as a free-flowing liquid which rapidly covers a considerable area around the point where damage has occurred. Most *C. rostrata* trees show signs of resin flow but are conspicuous by the absence of herbivore damage or pathogen attack on woody parts.

The volatile portions of the resins from a number of Kenyan species of *Commiphora* have recently been studied and have been found to consist mainly of monoterpenoids [6] or sesquiterpenoids [7]. There do not appear to be any reports in the literature concerning the volatile resin from *C. rostrata*. In this paper we describe the results of our study of a sample of the resin obtained from plants growing in Meru National Park and give some preliminary data on its potential importance in the defence of the plant.

RESULTS AND DISCUSSION

Gas chromatography revealed the presence of at least 30 components in the oil. Table 1 lists 24 from which mass spectra were obtained. Of the 22 that have been identified 18 were characterized by direct comparison to library mass spectra and co-injection with authentic samples on two capillary columns. The major components are 2-decanone, 2-undecanone and 2-dodecanone, which make up *ca* 65, 24 and 5% of the volatiles, respectively. 2-

Table 1. Compounds identified from *Commiphora rostrata* oil

Peak number	Compound	% in resin*	Identification
1	2-Octanone	t	MS, R_t
2	2-Nonanone	t	MS, R_t
3	2-Decanone	65.0	MS, R_t
4	Unknown	t	
5	Unknown	t	
6	3-Undecanone	t	MS, R_t
7	2-Undecanone	24.0	MS, R_t
8	2-Decanol	t	MS, R_t
9	2-Dodecanone	5.0	MS, R_t
10	2-Undecanol	t	MS, R_t
11	2-Tridecanone	t	MS, R_t
12	Tridecanal	t	MS, R_t
13	2-Dodecanol	t	MS, R_t
14	2-Tetradecanone	t	MS, R_t
15	Tetradecanal	t	MS, R_t
16	2,2-Dimethylnonanol	t	DMS
17	2-Pentadecanone	t	MS, R_t
18	Pentadecanal	t	MS, R_t
19	2,2-Dimethyldecanol	t	DMS
20	Hexadecanal	1.5	MS, R_t
21	2,2-Dimethylundecanol	t	DMS
22	Heptadecanal	t	MS, R_t
23	2,2-Dimethyldodecanol	t	DMS
24	Octadecanal	t	MS, R_t

*t = trace amount, less than 1% of resin.
MS = EIMS matching with library spectrum.
R_t = Identical retention time with authentic sample on two capillary columns.
DMS = EIMS of TMSi ether.

*Part 9 in the series 'Chemistry of the Burseraceae'. For Part 8 see ref [1].

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Octanone, 2-nonanone, 2-tridecanone, 2-tetradecanone and 2-pentadecanone occur at concentrations of less than 1% while the corresponding 3-undecanone was found only in trace amounts. Three secondary alcohols (C_{10}–C_{12} 2-alkanols) also occur at trace levels. Another series is made up of saturated long-chain aldehydes between C_{13} and C_{18} which occur in a ratio of *ca* 1:1:1:18:5:3, with hexadecanal constituting *ca* 1.5% of total volatiles.

A further four trace components (peaks 16, 19, 21, 23, in Table 1) formed another homologous series of aliphatic alcohols. One of these (peak 19) was isolated by preparative GC. Its EI mass spectrum showed a highest fragment ion at *m/z* 155, with no other major significant ions other than those typical of a long-chain hydrocarbon. No ion attributable to $[M-H_2O]^+$ could be detected. The alcohol was derivatized using bis-trimethylsilyl trifluoroacetamide (BSTFA), and the resulting TMSi ether analysed by GC/MS. The mass spectrum of the ether revealed an $[M-15]^+$ ion at *m/z* 243 (14.5%) and some spectra showed a weak $[M]^+$ at *m/z* 258 indicating that the original alcohol was 186 mu, corresponding to a molecular formula of $C_{12}H_{25}OH$.

Two significant features of the fragmentation of the TMSi ether were the major ion at *m/z* 103 $[CH_2{=}O{-}Si(Me)_3]^+$ and at *m/z* 155 (6%) for the ion also observed in the free alcohol. These observations suggest that the carbon β to the oxygen must be quaternary in nature. This would be consistent with loss of CH_2OH from the original alcohol to give *m/z* 155 and would explain the absence of facile loss of the elements of water. On this basis we suggest that peak 19 must be 2,2-dimethyl-1-decanol. The mass spectra of the TMSi ethers of peaks 16, 21 and 23, formed by derivatization of the whole extract, were similar to that of peak 19 showing $[M-15]^+$ and abundant *m/z* 103 ions. Available data on shorter chain analogues of 2,2-dimethylalkanols (2,2-dimethylpropanol, butanol, pentanol, hexanol and 2,2,4-trimethylpentanol) all show significant $[M-31]^+$ ions and the lack of $[M-18]^+$ ions. As far as we are aware the 2,2-dimethylalkanols reported here have not previously been isolated from a plant source. The mass spectra of other TMSi ethers in the whole extract were consistent with the presence of C_{10}–C_{12} 2-alkanols.

It seems probable that the volatile resin of *C. rostrata* plays a role in defence against potential pests and pathogens. It has already been noted that the bark is conspicuously free from damage due to either boring insects or browsing mammals. Observations of recently cut bark from which resin is flowing indicate that insects in the vicinity (ants, termites) immediately become excited and move rapidly away from the wound. The resin literally sprays or squirts from a cut or stress point (caused by bending) and this may have an overwhelming effect on attacking predators or pathogens. After a short time a white sticky substance forms at the wound site, perhaps due to polymerisation of the resin aldehydes, and this presumably acts to protect and prevent water loss from the wound.

The occurrence of large quantities of aliphatic ketones in exudates has previously been implicated in chemical defence. The wild tomato, *Lycopersicon hirsutum* f. *glabratum* C. H. Mull., has glandular trichomes rich in 2-tridecanone which is thought to be responsible for its resistance to the tobacco hornworm, *Manduca sexta* (L.), an important pest of the cultivated tomato [8–11]. 2-Tridecanone is also toxic to the larvae of the tomato fruitworm, *Heliothis zea* (Boddie) [9, 10, 12], and is reported to be an ant alarm pheromone [13]. 2-Undecanone, which is a lesser component of the trichomes of *L. hirsutum* f. *glabratum*, increases larval mortality of *Heliothis zea* when mixed with 2-tridecanone and will cause deformity and mortality in *H. zea* pupae either alone or combined with 2-tridecanone [12]. 2-Nonanone, 2-decanone, 2-dodecanone and 2-pentadecanone are also toxic to *Heliothis zea* [14] while 2-tridecanone and 2-undecanone have been found to be toxic to neonate larvae of the tomato pinworm and the beet armyworm [15]. 2-Nonanone has also been reported to be an alarm pheromone in ants, hornets and honeybees and to have a weak bactericidal activity [16].

Sufficient resin was available to allow some preliminary tests for antifungal activity. Screening of the whole resin and its three major components indicated considerable activity against *Aspergillus* and *Penicillium* species; *A. niger* proving to be particularly susceptible (Table 2). In addition to inhibition of fungal growth the resin also prevented synthesis of mycotoxins by *Aspergillus* species. In the case of *A. flavus*, while there was less than 10% reduction of growth the production of aflatoxin B_1 was completely inhibited.

EXPERIMENTAL

Plant material. Resin was collected from specimens of *C. rostrata* Engl. growing in the Meru National Park, Kenya. Small

Table 2. Antifungal and antimycotoxigenic properties of *Commiphora rostrata* resin and its major constituents (A = 2-decanone, B = 2-undecanone, C = 2-dodecanone, dose level employed, 5000 ppm)

	Whole resin		Individual components					
			A		B		C	
Test organism	GI*	MS†	GI	MS	GI	MS	GI	MS
Aspergillus flavus	50.0	—	59.4	—	7.4	—	17.9	—
Aspergillus niger	78.5	NT	74.6	NT	75.6	NT	53.2	NT
Aspergillus ocharceus	78.9	—	84.3	—	71.3	—	39.8	—
Penicillium sp. A	68.2	NT	63.5	NT	47.1	NT	66.3	NT
Penicillium sp. B	35.8	NT	25.2	NT	25.1	NT	56.1	NT

*GI = Growth inhibition expressed as percentage reduction compared with control.
†MS = Mycotoxin synthesis (— = negative, NT = not tested).

quantities of neat resin were obtained by bending or cutting twigs and allowing it to drip into glass vials which were capped with teflon-lined screw caps. Alternatively resin was washed into vials with small amounts of purified hexane. The vials were stored in an insulated container of dry ice for transportation to Nairobi.

Analysis of resin. Samples, diluted with hexane, were analysed by capillary GC on two columns: (A) 25 m × 0.25 mm id CP Sil 5 fused silica (Chrompack); $T_{initial}$ 90° (2 min), T_{final} 250°; rate 10°/min; N_2 at 20 cm/sec: (B) 30 m × 0.25 mm id Superox fused silica (Alltech Associates); $T_{initial}$ 60° (2 min), T_{final}180°; rate 5°/min; H_2 at 40 cm/sec. Prep. GC was performed on a 3m × 2 mm id Carbowax 20 M packed column operated at 180°.

GC–MS analyses were performed on a quadrupole instrument in the EI mode (70 eV) employing a 30m × 0.25 mm id CP Wax 51 fused silica column (Chrompack); $T_{initial}$ 60° (2 min), T_{final} 200°, rate 5°/min, He at 25 cm/sec. Scan time was 1 sec with mass range 35–320. Silylation reactions were carried out by the addition of 5 μl aliquots of bis-trimethylsilyl trifluoroacetamide (BSTFA with 1% TMCS), at room temp.

Anti-fungal testing. The extract was tested against five fungal strains, maintained on potato dextrose agar at 25° and subcultured every month. The organisms used were *Aspergillus flavus* (IMI 89717), *A. niger* (IMI 17454), *A. ochraceus* (IMI 13242), *Penicillium* sp. A (isolate from *Myrica gale*), *Pencillium* sp. B (isolate from *Rosmarinus officinalis*).

Malt extract broth was inoculated with fungal spores (washed in 0.01% v/v Tween 80, stored in sterile dist H_2O) to a final concn of *ca* 10^5/ml. The resin and its three major constituents were each added to flasks to give a final concn of 5000 ppm. All flasks were prepd in duplicate and placed on an orbital incubator, running at 25°, for 5 days. After this time wet and dry wt measurements were taken following filtration through Whatman GF/C filter papers.

Mycotoxigenesis was detected by spotting filtrate on silica gel 60 TLC plates and developing for 90 min in toluene–EtOAc–HCO_2H (6:3:1). Aflatoxin B_1 and ochratoxin A were run as stds. Visualization of the mycotoxins was by viewing under UV light.

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