

molecules of 1 and one of acetyl-CoA as summarized in Scheme 1.

EXPERIMENTAL

Dried, powdered, wood (3.75 kg) of *B. zeylanica* collected at Hasalaka, Sri Lanka, was successively extracted with hot light petroleum and hot C_6H_6 . The C_6H_6 extract (12.0 g) was transferred to a column of silica gel (Merck, 30–70 mesh) and eluted with C_6H_6 containing increasing amounts of $CHCl_3$. The C_6H_6 – $CHCl_3$ (49:1) eluates yielded (1), mp 155–156° (420 mg), C_6H_6 – $CHCl_3$ (4:1) gave (2), mp 223–224° (130 mg), both identified by comparison with authentic samples previously isolated [2, 3], and C_6H_6 – $CHCl_3$ (3:2) afforded broussonetine (3) as tiny crystals, mp 238–239° (from $CHCl_3$), $[\alpha]_D^{20} = 0^\circ$ (c 0.11; DMF); (Found: C, 71.3; H, 4.5; N, 7.5%; $[M]^+$, 372.111. $C_{22}H_{16}N_2O_4$ requires C, 70.5; H, 4.3; N, 7.5%; $[M]^+$, 372.1110); UV λ_{max}^{MeOH} nm (log ϵ): 252 (4.19) and 333 (3.70); $\lambda_{max}^{MeOH-NaOH}$ nm: 271 (4.29), 344 (3.99) and 386 (4.07); IR ν_{max}^{KBr} cm^{-1} : 3300, 1775, 1570, 1510, 1470, 1405, 1365, 1330, 1270, 1225, 1160, 1130, 1050, 1015, 930, 750 and 690; MS (70 eV) m/z (rel. int.): 372 $[M]^+$ (100%), 344 (2), 328 (2), 172 (47), 171.0687 (89, $C_{11}H_8NO$ requires 171.0684), 170 (30), 145 (18), 143 (28), 142 (16), 117 (15). When treated with Ac_2O (1.0 ml) and pyridine (2.0 ml) broussonetine (20 mg) gave a diacetate (4); crystals, mp 162–163° (C_6H_6 –petrol) (15.2 mg, 76%). (Found: $[M-CH_2CO]^+$, 414.1245. $C_{24}H_{18}N_2O_5$ requires 414.1215; IR ν_{max}^{KBr} cm^{-1} : 1775, 1755, 1601, 1591, 1500, 1470, 1410, 1370, 1310, 1210 *br*, 1180 *br*,

941, 771 and 755; 1H NMR (360 MHz, $CDCl_3$); δ 2.50 and 2.52 (each 3H, s, OAc), 2.68 (1H, *dd*, $J = 3.1$ and 17.9 Hz, H-2a), 3.18 (1H, *dd*, $J = 9.3$ and 17.9 Hz, H-2b), 4.50 (1H, *dt*, $J = 3.1$ and 9.3 Hz, H-3), 6.51 (1H, *dd*, $J = 0.7$ and 3.1 Hz, H-4), 7.12–7.64 (8H, *m*, ArH), 8.97 and 9.00 (each 1H, *d*, $J = 4.5$ Hz, H-2 and H-2'); MS (20 eV) m/z (rel. int.): 456 $[M]^+$ (5%), 414 (18), 372 (100), 172 (37), 171 (67), 170 (26), 145 (14), 143 (21) and 117 (7).

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SATIVANINE-C: A CYCLOPEPTIDE ALKALOID FROM THE BARK OF *ZIZYPHUS SATIVA**

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Key Word Index—*Zizyphus sativa*; Rhamnaceae; cyclopeptide alkaloid; sativanine-C.

Abstract—From the bark of *Zizyphus sativa* a previously undescribed 13 membered cyclopeptide alkaloid, sativanine-C has been isolated. The structure of this new compound was elucidated by spectroscopic methods, its transformation product and by chemical degradation.

INTRODUCTION

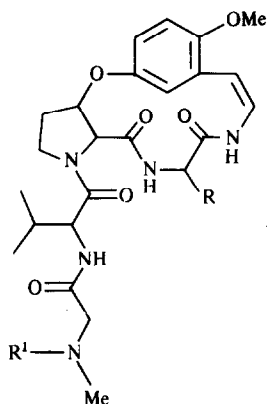
As a part of our extended studies on the alkaloids of the Rhamnaceae we recently reported the isolation and characterization of five cyclopeptide alkaloids from *Zizyphus sativa* [2]. Extensive chromatography of the

crude bases furnished a further previously unknown 13-membered cyclopeptide alkaloid (1). Its structure is related to nummularine-B (3) [3].

RESULTS AND DISCUSSION

The alkaloid was isolated from the polar fraction by TLC on silica gel. The molecular formula was determined by high resolution mass spectrometry as $C_{29}H_{43}N_5O_6$. The IR spectrum displayed characteristic secondary am-

*Part 34 in the series "The Alkaloids of Rhamnaceae". For Part 33 see ref. [1].



	R	R ¹
Sativanine - C (1)	$\begin{array}{c} \text{---CH---CH}_2\text{---Me} \\ \\ \text{Me} \end{array}$	H
N-Formylsativanine-C (2)	$\begin{array}{c} \text{---CH---CH}_2\text{---Me} \\ \\ \text{Me} \end{array}$	$\begin{array}{c} \text{O} \\ \\ \text{---C---H} \end{array}$
Nummularine - B (3)	---CH ₂ ---Ph	H

ide bands and absorptions attributed to a conjugated (C=C) double bond, an aryl ether and an *N*-methyl group. The UV spectrum showed absorption maxima at 320 and 258 nm characteristic of 13-membered cyclopeptide alkaloids [4].

The mass spectrum of 1 closely resembles that of nummularine-B (3), the main difference being that the [M]⁺ and all fragments which contain the amino acid bound to the nitrogen of the styrylamine function (b, c, d, e, f, g, h, i, j, p) are displaced by 34 mu to lower values. From this it could be concluded that 1 contains a C-6 amino acid (leucine or isoleucine) instead of phenylalanine as the ring bound amino acid. At *m/z* 86 the amine fragment [C₄H₉ -CH=NH₂]⁺ is observed. The base peak at *m/z* 58 shows the amine fragment a [C₃H₈N]⁺ which could be due to *N*-monomethylalanine or *N,N*-dimethylglycine. The elementary composition of all fragments was substantiated by high resolution mass measurements.

N-Formylsativanine-C (2) was prepared from 1 which proves that the end amino acid in 1 is *N*-monomethylalanine. The identity of the ring bound intermediate and end amino acids was proved to be isoleucine, valine and *N*-monomethylalanine, respectively, by PC comparison of the acid hydrolysate of 1 with the authentic samples. Thus structure 1 is confirmed for sativanine-C.

EXPERIMENTAL

Zizyphus sativa Gaertn was collected in Hazara district, Pakistan [5, 6]. Mps are uncorr. MS analysis was performed at 70 eV with evaporation of the sample in the ion source at ca 200°. TLC was carried out on silica gel HF₂₅₄ (Merck) and PC on Whatman No. 1 and 2043b (Scheicher & Schüll) papers.

Extraction. The crude alkaloids (6.6 g) were obtained by the

extraction of powdered bark (10 kg) with MeOH in the usual manner [7]. The mixture of crude alkaloids was fractionated on a column of 900 g silica gel M (Geb. Herrmann/Köln) eluting with increasingly polar CH₂Cl₂-MeOH mixtures into 15 fractions. The chromatographic separation was followed by UV monitoring, and collected fractions were analysed by TLC, proving in every case to be mixture of two or three main components. The fractions were separated into individual components by prep. TLC or CC.

Sativanine-C (1). 3.8 mg of 1 were obtained from fractions 12 and 13 [8] by repetitive TLC on silica gel using cyclohexane-EtOAc-MeOH (35:15:1) and CH₂Cl₂-MeOH (20:1) as solvent systems. Mp 113-114° (uncorr); IR ν_{max}^{CHCl₃} cm⁻¹: 3380, 1670, 1635 (sec. amide), 2835 (OMe), 2780 (NMe), 1610 (C=C), 1230 and 1040 (aryl ether). UV λ_{max}^{MeOH} nm: 320 and 258. MW (MS): 557.3200. Calc. for C₂₉H₄₃N₅O₆ 557.3213. MS 70 eV, *m/z* (rel. int.): 557 [M]⁺ (8), 58 [a] (100), 542 [b] (< 0.5), 500 [c] (0.5), 598 [d] (0.3), 457 [e] (9), 401 [f] (< 0.5), 400 [g] (< 0.5), 374 [h] (0.6), 373 [i] (2), 372 [j] (1), 185 [l] (1), 157 [m] (0.6), 195 [n] (0.7), 209 [p] (0.8), 259 [r] (0.6), 233 [s] (0.6), 216 [t] (2), 165 (C₉H₁₁NO₂, 11), 96 (C₅H₆NO, 2), 86 (C₅H₁₂N, 4), 72 (C₄H₁₀N, 7).

Formylation [9]. *N*-Formylsativanine-C (2) was prepared from 1 when reacted with HCO₂H-Ac₂O and kept overnight at room temp. The solvent was evaporated and the product purified by TLC in cyclohexane-Me₂CO (3:1). Mp 130-131°. MW (MS): 585.3177. Calc. for C₃₀H₄₃N₅O₇: 585.3163. MS *m/z*: 585 (C₃₀H₄₃N₅O₇, [M]⁺, 28), 114 (C₅H₈NO₂, 100), 86 (C₄H₈NO, 50), 58 (C₃H₈N, 37).

Hydrolysis. Compound 1 was heated in a sealed tube with 1 ml of 6 N HCl for 24 hr at 110°. Excess reagent was evaporated *in vacuo* and the residue taken up in H₂O for PC. The amino acids were identified by comparison with authentic samples using *n*-BuOH-HOAc-H₂O (4:1:5) [10] and *n*-BuOH-H₂O-Me₂CO-NH₃ (8:6:1:1) [11] as solvent systems and ninhydrin as spray reagent.

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THE VOLATILE CONSTITUENTS OF CAMPHORWEED, *HETEROTHECA SUBAXILLARIS*

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Key Word Index—*Heterotheca subaxillaris*; Asteraceae; essential oil; monoterpene; sesquiterpene.

Abstract—Forty-one mono- and sesquiterpenoids are identified from the leaf volatiles of *Heterotheca subaxillaris*. Affinities to related genera are briefly discussed.

INTRODUCTION

The objectives of the present study were to determine the identities and proportions of the principle volatile compounds accumulated in the leaves of *Heterotheca subaxillaris*. Although the leaf chemistry of many Asteraceae have been investigated, members of the tribe Astereae have received only limited attention [1]. These taxa appear to be characterized by a lack of sesquiterpene lactones or alkaloids, but commonly accumulate mono-, sesqui- and diterpenoids. The leaf chemistry of *H. subaxillaris* has been partially studied and several sesquiterpenoids have been previously identified [2–7].

RESULTS

The observed identity and proportion (% of total volatiles in sample 1, % of total volatiles in sample 2) of the volatile constituents were: 2-ethylfuran (0.02, 0.01), α -pinene (8.60, 0.15), camphene (2.44, 0.08), β -pinene (1.37, 0.16), sabinene (0.11, 0.04), myrcene (9.82, 0.37), α -terpinene (0.14, 0.13), limonene (5.57, 0.34), *trans*-2-hexenal + unknown (0.71, 0.07), *cis*-ocimene (0.16, 0.02), γ -

terpinene (0.11, 0.48), *trans*-ocimene (2.91, 0.60), *p*-cymene (0.71, 0.22), terpinolene (0.08, 0.14), *cis,cis*-allo-ocimene (0.07, 0.09), *cis*-3-hexenol (0.12, 0.01), *trans*-2-hexenol (0.03, 0.01), 1-octen-3-ol (0.38, 0.19), *trans*-sabinene hydrate (0.07, 1.44), α -cubebene (0.15, 0.09), camphor (1.25, 1.53), *cis*-sabinene hydrate (0.05, 1.69), bornyl acetate (5.20, 4.62), *trans*- α -bergamotene (5.46, 2.48), β -elemene (0.12, 0.65), terpinen-4-ol (0.98, 3.60), caryophyllene (11.07, 1.70), α -himachalene (tentative) (0.23, 0.12), unidentified sesquiterpene hydrocarbon (0.56, 0.77), α -humulene (0.61, 0.38), borneol (8.07, 9.84), germacrene D (5.68, 7.30), bicyclogermacrene (tentative) (1.08, 0.02), carvone (1.12, 1.23), γ -elemene (0.31, 0.87), δ -cadinene (1.59, 1.71), α -curcumene (0.55, 0.42), calamenene (0.31, 0.37), geranyl acetone (0.09, 0.21), nerolidol (0.09, 0.21), epicubenol (1.95, 6.87), α -cadinol (0.13, 0.91), unidentified sesquiterpene alcohol (1.98, 6.22), unidentified sesquiterpene alcohol (1.44, 8.12).

DISCUSSION

The subtribal boundaries in the Astereae are not well defined [8] and, although there is still not sufficient