graphed with PM 6,8-di-*C*-glucosylluteolin. The free compound 1 cochromatographed with 6,8-di-*C*-glucosylluteolin.

6,8-Di-*C*-glucosylchrysoeriol: PC $R_f \times 100$: 12, 49, 14, 68, 17, 66, 13. UV nm: MeOH, 340, 286 (sh), 275; MeO^-, 407, 337 (sh); $AlCl_3$, 388 (sh), 361, 282; $AlCl_3/HCl$, 385 (sh), 356, 282; NaOAc, 397, 321, 283. The PM ether produced M^+ 778 and the fragmentation pattern of a 6,8-di-*C*-hexosylluteolin and cochromatographed with PM 6,8-di-*C*-glucosylluteolin. The free compound cochromatographed with 6,8-di-*C*-glucosylchrysoeriol.

G. gnemon. Swertisin: UV, NMR, and R_f values for this compound have been published [2]. The previous identification was verified by cochromatography with authentic swertisin using conditions where *C*-glucosides separate from *C*-galactosides (activated Si gel TLC: EPWM).

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CONFIRMATION OF STRUCTURE OF THE FLAVONOL GLUCOSIDE TAMBULETIN

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Key Word Index—*Zanthoxylum alatum*; Rutaceae; tambuletin; 3, 5, 3′-trihydroxy-7, 4′-dimethoxy-8-*O*-β-D-glucopyranosylflavone; structure determination.

Abstract—The structure of tambuletin, present in seeds of *Xanthoxylum* species has been confirmed as the 8-glucoside of gossypetin 7, 4′-dimethyl ether.

INTRODUCTION

Zanthoxylum acanthopodium and *Z. alatum* (Rutaceae) are well-known Indian indigenous plants [1]. Bose and Bose [2] isolated two flavonols—tambulin and tambulol— from *Z. acanthopodium* and assigned the structure of 5, 7-dihydroxy-3, 8, 3′-trimethoxy or 5, 7-dihydroxy-6, 8, 4′-trimethoxy flavone for tambulin. Later Balakrishna and Seshadri [3] isolated tambuletin (in the place of tambulin and tambulol) from the same plant and characterized it as 8-*O*-methylherbacetin. They also considered, on synthetic grounds, that tambulin was 7, 8, 4′-tri-*O*-methylherbacetin. Subsequently, Harborne *et al.* [4]. showed that tambuletin isolated by Balakrishna and Seshadri [3] was a glycoside with 3, 5, 3′-trihydroxy-7, 4′-dimethoxy-8-*O*-glucosyl or (less likely) 3, 5, 3′-trihydroxy-8, 4′-dimethoxy-7-*O*-glucosylflavone structure; they also suggested that tambulin could be a gossypetin derivative. The structure of tambulin was later unequivocally established as 7, 8, 4′-tri-*O*-methylherbacetin by two independent groups [5, 6]. Chatterjee *et al.* [6] isolated tambulin from *Z. alatum* and tambulol from *Z. acanthopodium* and found that tambulol and tambuletin were identical. They concluded, without offering any further convincing evidence, that tambuletin was 3, 5, 3′-trihydroxy-7, 4′-dimethoxy-8-*O*-glucosylflavone. We now confirm that tambuletin, isolated from *Z. alatum* for the first time, is indeed 3, 5, 3′-trihydroxy-7, 4′-dimethoxy-8-*O*-β-D-glucopyranosylflavone.

RESULTS AND DISCUSSION

The flavonoid obtained from the acetone extract of the defatted seeds of *A. alatum* was identified as tambulin (3, 5-dihydroxy-7, 8, 4′-trimethoxyflavone) [5, 6] by mp and mmp, λ_{max}, MS, demethylation, methylation and co-TLC with an authentic sample.

The flavonol glycoside, obtained from the MeOH extract of the marc (after Me_2CO), when crystallized twice from EtOAc yielded yellow needles, $C_{23}H_{24}O_{13}$, mp 268–269°; hepta-acetate, $C_{37}H_{38}O_{20}$, mp 142–143°, identified as tambuletin [4, 6] by λ_{max}, products of acid as well as alkali treatment and co-TLC with an authentic sample. Tambuletin yielded an aglycone and D-glucose in 1 : 1 ratio on hydrolysis with acid and enzyme (β-glucosidase) and consumed 2 mol of HIO_4 yielding 1 mol of HCO_2H, showing it to be a β-D-glucopyranoside; the β-configuration was further supported [7] by the ^{1}H NMR spectrum of its hepta-acetate (H-1 signal of glucose was a well-defined doublet, $J = 8$ Hz, at δ 5.08).

The aglycone, $C_{17}H_{14}O_8$ was purple under UV and UV/NH_3 while the glycoside was yellow and on exposure to NH_3 it changed to green while the glycoside gave a stable yellow colour. The glycoside on permethylation and hydrolysis yielded 3, 5, 7, 3′, 4′-pentamethoxy-8-hydroxyflavone (identified by mp and co-TLC with the sample obtained from gossypin [8] on similar treatment) and 2, 3, 4, 6-tetra-*O*-methylglucose. The position of the free OH in the partial Me ether was further established to be at C–8 (and not at C–7) by its nuclear reduction [9] to form penta-*O*-methylquercetin. The C_6D_6-induced shift of both OMe protons (3.95–3.61 and 3.92–3.58 δ) of tambuletin acetate also supported the presence of 7–OMe, since 8–OMe is not expected to show any shift due to the absence of an *o*-proton [10]; the position of the other OMe was fixed at C–4′ from the formation of 3-hydroxy-4-methoxy-benzoic acid by alkali fission (identified by co-TLC with authentic 3-hydroxy-4-methoxy- and 3-methoxy-4-hydroxy-benzoic acids). Thus, the structure of tambuletin was unequivocally established as 3, 5, 3′-trihydroxy-7, 4′-dimethoxy-8-*O*-β-D-glucopyranosylflavone (7, 4′-di-*O*-methylgossypin). The present isolation of tambuletin from *Z. alatum* together with its reisolation from *Z. acanthopodium* by Chatterjee *et al.* [6] finally removes any doubt [11] that the sample examined by Harborne *et al.* [4] might be from a different plant source.

EXPERIMENTAL

Authentic seeds of *Z. alatum* (supplied by United Chemical and Allied Products, Calcutta) were defatted with petrol (60–80°) and extracted with hot Me_2CO followed by MeOH.

Tambulin. Me_2CO, mp 206–207°, λ_{max} (nm) (MeOH) 273, 325, 380; (+NaOAc) 275, 325, 380 and (+$AlCl_3$) 275, 355, 440; MS: (*m*/*z*) 344 ($C_{18}H_{16}O_7$, M^+, 87%), 329 ($M^+ - Me$, 100), 315, 301, 168, 154, 135 and 107; diacetyltambulin, mp 160–162°; demethylation (Ac_2O + HI) gave herbacetin.

Tambuletin. MeOH, yellow solid, mp 268–270° (EtOAc), λ_{max}. (MeOH and +NaOAc) 255, 270, 380 and (+$AlCl_3$) 265, 345, 435; MS: 346 ($C_{17}H_{14}O_8$, aglycone, 100%), 332, 314 and 169; demethylation gave gossypetin.

Permethylation and hydrolysis [12] *of tambuletin and gossypin.* 3, 5, 7, 3′, 4′-Pentamethoxy-8-hydroxyflavone (mp 196–197°; nuclear reduction [9]: 3, 5, 7, 3′, 4′-pentamethoxyflavone, mp and mmp 150–151°) and 2, 3, 4, 6-tetra-*O*-methyl glucose (co-TLC, SiO_2, EtOAc–$CHCl_3$, 1 : 1).

Tambuletin hepta-acetate. ($Ac_2O + C_5H_5N$, 24 hr), mp 142–143°, ^{1}H NMR (400 MHz, $CDCl_3$ and $CDCl_3$–C_6D_6, 2 : 1, δ values, TMS internal; values in $CDCl_3$–C_6D_6 alone given): 1.75, 1.83, 1.87 and 1.89 (*s* each, 3 H each, 6″–, 4″–, 3″– and 2″–COC $\underline{\mathrm{Me}}$); 2.19, 2.22 and 2.35 (*s* each, 3H each, 3′–, 3– and 5–OCO$\underline{\mathrm{Me}}$); 3.58 and 3.61 (*s* each, 3H each, 4′– and 7–O$\underline{\mathrm{Me}}$); 3.50 (*m*, 1H, 5″–$\underline{\mathrm{H}}$); 3.92 (*dd*, $J = 12$ and 2.5 Hz, 1H, 6″–$\underline{\mathrm{H}}$); 4.16 (*dd*, $J = 12$ Hz and 6Hz, 1H, 6″–$\underline{\mathrm{H}}$); 5.08 (*d*, $J = 8$ Hz, 1H, 1″–$\underline{\mathrm{H}}$); 5.18 (*dd*, $J = 9$ Hz, 1H, 4″–$\underline{\mathrm{H}}$); 5.31 (*dd*, $J = 9$ Hz, 1H, 3″–$\underline{\mathrm{H}}$); 5.42 (*dd*, $J = 8$ Hz, 1H, 2″–$\underline{\mathrm{H}}$); 6.47 (*s*, 1H, 6–$\underline{\mathrm{H}}$); 6.88 (*d*, $J = 9$ Hz, 1H, 5′–$\underline{\mathrm{H}}$); 7.78 (*d*, $J = 2$ Hz, 1H, 2′–$\underline{\mathrm{H}}$) and 7.83 (*dd*, $J = 9$ Hz and 2 Hz, 1H, 6′–$\underline{\mathrm{H}}$). MS: 802 ($C_{37}H_{38}O_{20}$, M^+, 0.4), 760 ($M^+ - CH_2CO$, 1.3), 472 (aglycone triacetate, 1.8), 430 ($472 - CH_2CO$, 8), 388 ($472 - 2CH_2CO$, 20), 346 (aglycone, $C_{17}H_{14}O_8$, 18), 331 ($346 - Me$, 32), 169 (trihydroxy A-ring fragment + H, 100), 139 (5) and 127 (10).

Acid and β-glucosidase hydrolysis [7]. Tambuletin aglycone (mp 255–256° d, λ_{max} 260, 275, 340, 385; MS: similar to tambuletin) and D-glucose (co-TLC).

Paper chromatography. Tambulin, tambuletin and tambuletin aglycone had R_f: ($\times 100$, Whatman No. 1, 28°, in the order) 0, 3, 0 (H_2O); 5, 15, 6 (15% HOAc); 57, 57, 46 (50% HOAc); 95, 47, 82 (BAW); 95, 86, 90 (phenol); 64, 68, 65 (Forestal) and 91, 45, 61 (*t*-BAW).

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ANTIMICROBIAL ALKALOIDS FROM *BOEHMERIA CYLINDRICA*

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Key Word Index—*Boehmeria clyindrica*; Urticaceae; alkaloids; cryptopluerine; julandine; 3,4-dimethoxy-ω-(2′-piperidyl)acetophenone; antimicrobial activity.

Abstract—Investigation of extracts of *Boehmeria cylindrica* resulted in identification of cryptopleurine and 3,4-dimethoxy-ω-(2′-piperidyl)acetophenone, known alkaloids, as agents responsible for intense activity against *Candida albicans*. Julandine, the secophenanthroquinolizidine alkaloid related to cryptopleurine, was also active but not definitely identified in the extracts examined.

In connection with our screening program for antimicrobial agents from higher plants, we wish to report the surprisingly intense antimicrobial activity of alkaloidal constituents of *Boehmeria cylindrica* (L.) Sw (Urticaceae), of Kansas origin. The crude alcoholic extracts of this plant were active *in vitro* in an agar-dilution assay at less than 1000 μg/ml, so bioassay directed fractionation studies were initiated following our usual scheme (cf. [1]). The anticandidal activity was found to reside in the crude alkaloidal fraction (MIC = 6 μg/ml), but the relatively small amount of plant material available precluded detailed fractionation. Accordingly, we turned to the more abundant *B. cylindrica* var. *drummondiana* Wedd., whose spectrum and potency paralleled those of the original species.

Interestingly, autobiographical techniques involving TLC strips of *B. cylindrica* alkaloids laid on seeded lawns of *C. albicans* in agar plates demonstrated two well-separated zones of inhibition in areas of the strips where UV light, various spray reagents and iodine vapors failed to reveal the presence of definite components. These findings showed that too little of the active constituents were present for detection in the usual fashion. Due to the generosity of Dr. J. A. Lamberton, we were able to make a side-by-side comparison, using TLC bioautographs, with the following known *Boehmeria* alkaloids: the cytotoxic agent cryptopleurine (**1**), synthetic [2] julandine (**2**), and 3,4-dimethoxy-ω-(2′-piperidyl)acetophenone (**3**). The TLC spots for **2** and **3** were rather close to one another and paralleled the inhibition zone of lower R_f. Compound **1** paralleled the inhibition zone of higher R_f. Prep. TLC finally resulted in the isolation of small amounts of incompletely pure **1** and **3**, accounting for about 0.00002% of the weight of the dried plant material, while **2** was not found in isolable amounts. The isolated compounds were identical to the authentic samples of **1** and **3** both by TLC and by HPLC spiking experiments.

Agar dilution assays of **1**, **2**, and **3** revealed minimum inhibitory concentration values against *C. albicans* of 0.1, 12.5 and 3.12 μg/ml, respectively. Plant-derived antimicrobial agents, in our experience,

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