collected in late April, and voucher specimens have been deposited at the Herbarium, Cairo University.

Methods. The plant material was extracted with 70% EtOH and concd under red. pres. The extract was fractionated using elution techniques on PC. Fractions were subjected to detailed studies according to standard methods [10, 11].

Kaempferol and quercetin 3-glucuronide-7-glucosides. The glycosides gave kaempferol and quercetin respectively on acid hydrolysis as well as glucose and glucuronic acid in both cases. Mild acid hydrolysis gave rise to the corresponding 7-glucosides for both glycosides and enzymatic hydrolysis with β-glucosidase gave rise to the corresponding 3-glucuronides of both flavonols. The UV data confirmed that positions 3 and 7 were occupied in both glycosides. R_f values for kaempferol and quercetin 3-glucuronide-7-glucosides and quercetin 3-glucuronide (as reference), respectively, are as follows: BAW = 20, 16, 39; PhOH = 20, 13, 16; H_2O = 63, 48, 23; 15% HOAc = 54, 48, 28.

Acknowledgement—I thank Miss Salwa A. Kawashty for technical assistance.

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Phytochemistry, Vol. 24, No. 2, pp. 372–374, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

ALKALOIDS FROM *HAPLOPHYLLUM BUXBAUMII*

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(*Received* 17 *May* 1984)

Key Word Index—*Haplophyllum buxbaumii*; Rutaceae; alkaloids; kokusaginine; skimmianine; γ-fagarine; 4,5,6-trimethoxyfuroquinoline; 4,5,7-trimethoxyfuroquinoline; *N*-hydroxymethylflindersine; justicin B.

Abstract—In addition to the known alkaloids kokusaginine, skimmianine, γ-fagarine and a lignan, justicin B, the aerial parts of *Haplophyllum buxbaumii* afforded three new alkaloids: 4,5,6-trimethoxyfuroquinoline, 4,5,7-trimethoxyfuroquinoline and *N*-hydroxymethylflindersine. The structures of the known and the new compounds were assigned by spectral methods.

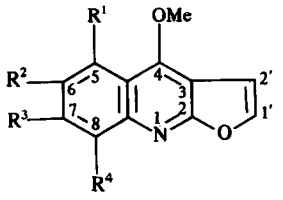
INTRODUCTION

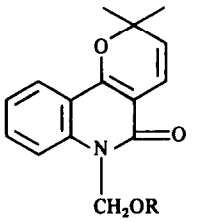
In previous studies, quinoline alkaloids [1–5], coumarins [6–8] and lignans [9–11] have been isolated from *Haplophyllum* species. In continuation of the investigation of the genus *Haplophyllum* [12], in addition to the known compounds kokusaginine [1], skimmianine [2], γ-fagarine [5] and justicin B [13, 14], we report here the isolation of three new alkaloids, two of which are furoquinolines and one is an angular pyranoquinoline type. The structures of the known and the new compounds were established by spectral methods.

RESULTS AND DISCUSSION

Benzene–chloroform extracts of the aerial parts of *Haplophyllum buxbaumii* subsp. *buxbaumii* yielded the known alkaloids kokusaginine (**1**), skimmianine (**2**), γ-fagarine (**3**), a lignan, justicin B (**4**), as well as the new alkaloids 4,5,6-trimethoxyfuroquinoline (**5**), 4,5,7-trimethoxyfuroquinoline (**6**) and *N*-hydroxymethylflindersine (**7**). The known compounds were identified by comparing their spectra to those in the literature and TLC comparison with standard samples in the case of **1** and **3**.

In the mass spectrum of **5**, the $[M]^+$ at *m/z* 259 indicated the molecular formula $C_{14}H_{13}O_4N$. The UV and IR spectra were similar to known furoquinoline alkaloids (**1–3**) indicating the same type of compound (see Experimental). In the ^1H NMR spectrum of **5**, three methoxyl singlets were present at δ4.43 (C_4-OMe), 4.10 (C_5-OMe) and 4.05 (C_6-OMe) as well as four proton doublets in the aromatic region. Since two of these protons at δ7.05 (1H, *d*, *J* = 2.5 Hz, H-1′) and 7.57 (1H, *d*,





1 $R^1 = R^4 = H$; $R^2 = R^3 = OMe$

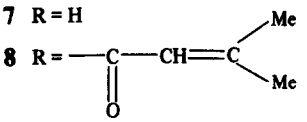
2 $R^1 = R^2 = H$; $R^3 = R^4 = OMe$

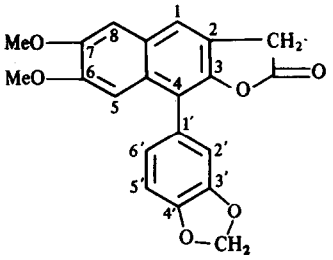
3 $R^1 = R^2 = R^3 = H$; $R^4 = OMe$

5 $R^1 = R^2 = OMe$; $R^3 = R^4 = H$

6 $R^1 = R^3 = OMe$; $R^2 = R^4 = H$

7 R = H

8 R = 



J = 2.5 Hz, H-2') clearly showed the furan ring protons, the other two should correspond to the A-ring protons at δ7.65 (1H, *d*, J = 8 Hz, H-8) and 7.40 (1H, *d*, J = 9 Hz, H-7) indicating that the two methoxyl groups should be at C-5 and C-6. The alternative positions for the methoxyl groups could be at C-7 and C-8, but in the mass spectrum of **5** the lack of $[M-1]^+$ and $[M-29]^+$ peaks showed that there could be no methoxyl group at C-8 [5]. Also, comparison with skimmianine on TLC plates showed that these were different compounds. The presence of a C-4 methoxyl group was confirmed by the *m/z* 244 $[M-15]^+$ and 216 $[M-43]^+$ peaks [5, 15]. Therefore the compound should have the structure given as **5**.

The UV, IR and mass spectra of compound **6** were similar to those of **5**. The only difference was in its ^{1}H NMR spectrum, in the aromatic region: δ7.05 (1H, *d*, J = 2.5 Hz, H-1'), 7.57 (1H, *d*, J = 2.5 Hz, H-2') showed the furan ring protons while the other protons at δ7.65 (1H, *d*, J = 2.5 Hz, H-8) and 7.08 (1H, *d*, J = 2.5 Hz, H-6) indicated their *meta* positions relative to one another. The lack of $[M-1]^+$ and $[M-29]^+$ peaks as in the mass spectrum of **5** and the presence of $[M-15]^+$ and $[M-43]^+$ peaks indicated that **6** should be 4,5,7-trimethoxyfuroquinoline.

In the mass spectrum of **7**, the $[M]^+$ at *m/z* 257 indicated the molecular formula $C_{15}H_{15}O_3N$, its UV and IR spectra being similar to those of flindersine [12]. The base peak in its mass spectrum at *m/z* 242 $[M-15]^+$ corresponds to the similar peak of flindersine (*m/z* 212 $[M-15]^+$), the peak at *m/z* 227 $[M-CH_2O]^+$ shows the flindersine part of **7**, *m/z* 212 $[M-CH_2O-Me]^+$ also indicates the flindersine skeleton. The basic hydrolysis of haplophylline (**8**) [12] yielded compound **7** (TLC comparison in $CHCl_3$–EtOH, 19:1). This new alkaloid could be an artefact derived from haplophylline during extraction.

EXPERIMENTAL

The plant material was collected from southern Turkey (Pozanti-Kamişli) in June 1983. It was identified by Dr. E. Tuzlaci (Istanbul). A voucher specimen has been deposited at the Herbarium of the Faculty of Pharmacy, University of Istanbul (ISTE 50888).

The dried and powdered stems and leaves of *H. buxbaumii* (600 g) were successively extracted with C_6H_6, $CHCl_3$ and EtOH in a Soxhlet. Since the C_6H_6 and $CHCl_3$ extracts showed the same alkaloids on TLC plates, they were combined.

Isolation of alkaloids. The same isolation method used in a previous paper [12] was also applied here. The combined C_6H_6 + $CHCl_3$ extracts were evapd under vacuum to dryness. The residue (15 g) was dissolved in $CHCl_3$, 5% NH_4OH was added, the mixture was concd to a small vol. and exhaustively extracted with $CHCl_3$. The combined $CHCl_3$ extracts were washed with H_2O, dried (Na_2SO_4) filtered and concd under vacuum. The $CHCl_3$ concentrate was extracted with 5% HCl until no further alkaloid was obtained. The aq. acid phase was made alkaline (conc. NH_4OH), extracted with $CHCl_3$, washed with H_2O, dried (Na_2SO_4), filtered and concd to dryness *in vacuo* to yield 1.2 g of crude alkaloid mixture (yield 0.2%). The alkaloid mixture was chromatographed on a neutral (activity III) Al_2O_3 column (3 × 50 cm); elution of the column was started with $CHCl_3$ and continued by gradient addition with EtOH. Alkaloids were further separated and purified by prep. TLC using mixtures of $CHCl_3$–C_6H_6–EtOH (8:5:2) as developing solvents.

4,5,6-Trimethoxyfuroquinoline (**5**). Amorphous. UV λ_{max}^{EtOH} nm: 334, 320, 310, 297 (sh), 252 (sh), 245, 209. IR ν_{max}^{KBr} cm^{-1}: 3110, 1620, 1580, 1510, 1480, 1450, 1390, 1370, 1265, 1210, 1150, 1095,

970, 940. ^{1}H NMR ($CDCl_3$): given in the text. MS *m/z* (rel. int.): 259 $[M]^+$ (100), 244 $[M-Me]^+$ (40), 229 $[M-2\times Me]^+$ (92), 216 $[M-CO-Me]^+$ (22), 214 $[M-3\times Me]^+$ (25), 200 $[M-COH-2\times Me]^+$ (50), 186 $[M-3\times Me-CO]^+$ (25). (Found: C, 64.95; H, 5.07; N, 5.41. $C_{14}H_{13}O_4N$ requires: C, 64.86; H, 5.02; N, 5.40%.)

4,5,7-Trimethoxyfuroquinoline (**6**).·Amorphous. UV λ_{max}^{EtOH} nm: 334, 320, 309, 296 (sh), 251 (sh), 245, 210. IR ν_{max}^{KBr} cm^{-1}: 3100, 1620, 1580, 1500, 1480, 1445, 1390, 1370, 1270, 1210, 1150, 1080, 970. ^{1}H NMR ($CDCl_3$): given in the text. MS *m/z* (rel. int.): 259 $[M]^+$ (100), 244 $[M-Me]^+$ (45), 229 $[M-2\times Me]^+$ (90), 216 $[M-CO-Me]^+$ (22), 214 $[M-3\times Me]^+$ (30). (Found: C, 64.92; H, 5.05; N, 5.42. $C_{14}H_{13}O_4N$ requires: C, 64.86; H, 5.02; N, 5.40%.)

N-Hydroxymethylflindersine (**7**). Amorphous. UV λ_{max}^{EtOH} nm: 366, 345, 320, 303 (sh), 252 (sh), 224. IR ν_{max}^{KBr} cm^{-1}: 3300, 3010, 2920, 1650, 1610, 1580, 1500, 1460, 1380, 1220, 1180, 1130, 1070, 970, 850. MS *m/z* (rel. int.): 257 $[M]^+$ (60), 242 $[M-Me]^+$ (100), 227 $[M-CH_2O]^+$ (45), 212 $[M-CH_2O-Me]^+$ (70).

Justicin B (**4**). UV λ_{max}^{EtOH} nm: 350 (sh), 315, 295, 258. IR ν_{max}^{KBr} cm^{-1}: 3000, 2960, 2830, 1750, 1615, 1590, 1500, 1475, 1430, 1370, 1330, 1260, 1235, 1220, 1150, 1030, 1000, 925, 760. ^{1}H NMR ($CDCl_3$): δ 7.72 (1H, *s*, H-1), 7.18 (1H, *s*, H-5*), 7.1 (1H, *s*, H-8*), 6.96 (1H, *d*, J = 9 Hz, H-5'), 6.8 (1H, *dd*, J = 9 and 2.5 Hz, H-6'), 6.85 (1H, *d*, J = 2.5 Hz, H-2'), 5.37 (2H, *d*, J = 1 Hz, CH_2 of lactone), 6.1 (1H, *d*, J = 1 Hz, O–CH(H)–O), 6.05 (1H, *d*, J = 1 Hz, O–CH(H)–O), 3.81 (3H, *s*, C_6-OMe†), 4.05 (3H, *s*, C_7-OMe†). (Signals marked with * and † are interchangeable.) MS *m/z* (rel. int.): 364 $[M]^+$ (100), 335 $[M-COH]^+$ (10), 319 $[M-2\times Me-CH_2]^+$ (15), 305 $[M-59]^+$ (20), 277 $[M-59-28]^+$ (25). (Found: C, 69.27; H, 4.42·$C_{21}H_{16}O_6$ requires: C, 69.23; H, 4.39%.)

Hydrolysis of haplophylline (**8**). **8** (4 mg) dissolved in EtOH was refluxed with 5 ml 5% NaOH in EtOH for 3 hr. The reaction mixture was diluted with H_2O, extracted with $CHCl_3$, dried (Na_2SO_4), filtered and evapd to dryness.

Acknowledgements—I thank Professor Dr. F. Bohlmann (Berlin) for the 400 MHz ^{1}H NMR spectra and Professor T. J. Mabry (Austin-Texas) for the mass spectra.

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