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Thapsakins: possible biogenetic intermediates towards insecticidal cyclopenta[*b*]benzofurans from *Aglaia edulis*

Markus Bacher[a], Otmar Hofer[a], Günter Brader[b], Srunya Vajrodaya[b, 1], Harald Greger[b,*]

[a]*Institute of Organic Chemistry, University of Vienna, Währingerstrasse 38, A-1090, Wien, Austria*
[b]*Comparative Phytochemistry Department, Institute of Botany, University of Vienna, Rennweg 14, A-1030, Wien, Austria*

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Abstract

Nine new flavaglines, cyclopenta[*bc*]benzopyrans (thapsakins) and benzo[*b*]oxepines (thapoxepines), were isolated from the lipophilic root extract of *Aglaia edulis* together with two known cyclopenta[*b*]benzofurans, aglaroxin A and pannellin. The structures were established on the basis of extensive use of NMR spectroscopic methods (C,H-COSY, NOESY, HMBC, lanthanide induced shifts). Aglaroxin A and pannellin exhibited the strongest insect toxicity toward neonate larvae of *Spodoptera littoralis*. Possible biogenetic connections between the three skeletal types are outlined and chemosystematic implications of flavagline formation are discussed.

Keywords: *Aglaia edulis*; Meliaceae; Cyclopenta[*b*]benzofurans; Cyclopenta[*bc*]benzopyrans; Benzo[*b*]oxepines; Flavaglines; Biosynthesis; Insect toxicity; *Spodoptera littoralis*

1. Introduction

From the many phytochemical reports on plants in the family Meliaceae, aromatic compounds with a cyclopenta[*b*]benzofuran skeleton represent a typical chemical character of the genus *Aglaia* (Nugroho et al., 1997a,b; Cui et al., 1997; Brader et al., 1998). Based on literature and unpublished results from our laboratory, they are responsible for the pronounced insecticidal activity of many different species (Nugroho et al., 1997a,b; Brader et al., 1999). Considering their co-occurrence with cyclopenta[*bc*]benzopyran derivatives in *A. argentea* Blume and *A. forbesii* King, their basic structure was suggested to be derived from a flavonoid nucleus linked to a cinnamic acid moiety (Dumontet et al., 1996). With respect to their common biogenetic origin and restricted occurrence to the genus *Aglaia*, we recently suggested naming this class of compounds flavaglines (Brader et al., 1998).

In continuation of our current screening for biologically active compounds, we now found very high insecticidal activities in the lipophilic root extract from *A. edulis* (Roxb.) Wall. (Thai: Langsat Khao), collected in southwest Thailand. This was in contrast to our previous finding in that species, where no insect toxicity could be detected at all (Brader et al., 1998). The species is known to be variable and, in Thailand, Pannell (1992) attempted to recognize two separate species, but was not able to do so because of the occurrence of intermediates between them. Compared with the crude extracts of many other *Aglaia* species, already prepared for our current screening, the HPLC-diode array analysis of *A. edulis* showed UV-spectra similar to those recently obtained from *A. elaeagnoidea* (A. Juss) Benth (Brader et al., 1998).

In addition to the major flavagline aglaroxin A (**1**), only known so far from a patent (Ciba-Geigy, 1996), and small amounts of the recently published pannellin (**2**) (Brader et al., 1998), all the other compounds iso-

* Corresponding author.
[1] Permanent address: Department of Botany, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

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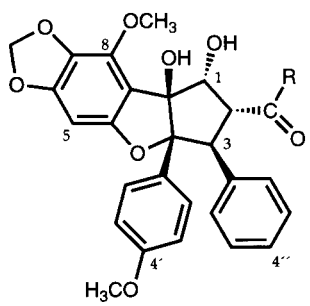
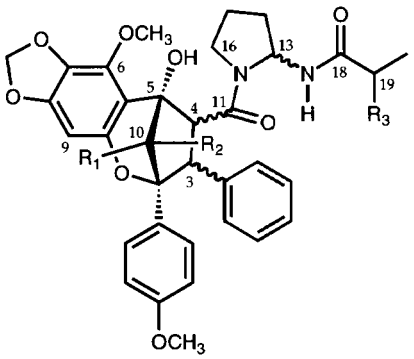
lated proved to be new, containing either a benzo[*bc*]-pyran (**3**–**8**) or a benzo[*b*]oxepine skeleton (**9**–**11**) as basic structures. Common to all is the same substitution pattern of the aromatic rings. According to the place of collection, near Thap Sakae in southwest Thailand, the compounds with a benzo[*b*]pyran moiety were named thapsakins (**3**–**8**) and those with a benzo[-*b*]oxepine skeleton thapoxepines (**9**–**11**) Call structures are shown in Scheme 1). Bioassays with the polyphagous pest insect *Spodoptera littoralis* (Lepidoptera, Noctuidae) revealed a pronounced insect toxicity for the cyclopenta[*b*]benzofuran derivatives aglaroxin A (**1**) and pannellin (**2**), whereas the two major compounds thapsakin A 10-*O*-acetate (**6**) and thapoxepine A (**9**) with a benzo[*bc*]pyran or benzo[*b*]oxepine skeleton, respectively, did not show marked insecticidal activity (Table 3). In the present paper we report the isolation and structure elucidation of the new derivatives as well as their insecticidal activities against *S. littoralis*. Scheme 1

λ_{max} towards 304 nm for **7** and **8**; and (iii) different spectra with λ_{max} at 280 nm and two characteristic shoulders at 335 and 297 nm for **9**–**11**. The IR spectra of compounds **3**–**11** are characterized by a sharp band at 3430 to 3436 cm^{-1} ($CHCl_3$ or CCl_4) indicating the N–H vibration of secondary amides. Typical bands at 1754 to 1760 cm^{-1} ($CHCl_3$ or CCl_4) and 1216 to 1223 cm^{-1} (CCl_4) in the major compounds **6** and **9** are indicative for an ester function at position C-10, whereas an oxo group at the same position in **7** and **8** is characterized by weaker bands at 1746 cm^{-1}. In accordance with a recent patent (Ciba-Geigy, 1996), the ^1H and ^{13}C NMR data of compound **1** were in favor of the cyclopenta[*b*]benzofuran derivative aglaroxin A, showing a methoxy and a methylenedioxy group at ring A, and a simple dimethyl amide at position 2. The ^{13}C NMR shift values of **1** agree perfectly with the data listed in the patent, however, the ^{13}C NMR resonances were listed without any assignments (Ciba-Geigy, 1996). In Section 3 we have therefore included



1 R = $N(CH_3)_2$

2 R = OCH_3



3 R_1 = H, R_2 = OH, R_3 = CH_3, H-3ß, H-4α;
3A ... 13*S*; **3B** ... 13*R*

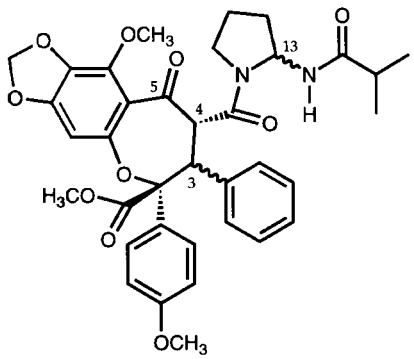
4 R_1 = OH, R_2 = H, R_3 = CH_3, H-3β, H-4α, 13*S*

5 R_1 = H, R_2 = OH, R_3 = CH_2-CH_3, H-3α, H-4ß, 13*S*

6 R_1 = H, R_2 = $OCOCH_3$, R_3 = CH_3, H-3α, H-4ß, 13*S*

7 R_1 = R_2 = O, R_3 = CH_3, H-3α, H-4ß, 13*S*

8 R_1 = R_2 = O, R_3 = CH_3, H-3ß, H-4α, 13*S*



9 R = CH_3, H-3α, H-4ß;
9A ... 13*S*; **9B** ... 13*R*

10 R = CH_2-CH_3, H-3α, H-4ß, 13*S*

11 R = CH_3, H-3β, H-4α;
11A ... 13*S*; **11B** ... 13*R*

2. Results and discussion

HPLC UV diode-array analysis of the lipophilic root extract of *A. edulis* revealed a preponderance of three compounds (**1**, **6**, **9**), which were isolated by preparative MPLC together with eight minor constituents. Based on characteristic UV spectra the compounds belong to three different types: (i) the already known type with λ_{max} at 298 nm (Brader et al., 1998) for compounds **2**–**6**; (ii) similar spectra with a slight shift of the completely assigned ^1H and ^{13}C NMR data, based on C,H-COSY and comparison with previous data [e.g. pannellin (**2**) (Brader et al., 1998)].

Compounds **3**–**6** showed closely related ^1H and ^{13}C NMR characteristics: apart from the typical resonances for a 6-methoxy-7,8-methylenedioxy substituted aromatic ring A, the *para*-substituted ring B (Ar′) and the simple phenyl ring C (Ar″), already described for pannellin (**2**) (Brader et al., 1998), they have amide links from the carboxylic group of the flavagline skel-

Table 1
^{1}H-NMR data for compounds **3**–**11**[a]

	3A/B[b]	**4**	**5**	**6**	**7**	**8**	**9A/B**[b]	**10**	**11A/B**[b]
3	4.76/4.82 *d*	4.28 *d*	4.50 *d*	4.56 *d*	4.95 *d*	4.53 *d*	5.11/5.23 *d*	5.13 *d*	5.30/5.28 *d*
4	3.99/3.85 *d*	3.88 *d*	4.17 *d*	4.12 *d*	4.51 *d*	4.40 *d*	4.55/4.40 *d*	4.53 *d*	4.64/4.38 *d*
9	6.15/6.20 *s*	6.11 *s*	6.15 *s*	6.13 *s*	6.38 *s*	6.39 *s*	6.51/6.59 *s*	6.50 *s*	6.62/6.65 *s*
10	4.25/4.20 *d*	5.10 *d*	4.76 *s*	6.13 *s*	–	–	–	–	–
13	5.97/5.50 *m*	6.10 *br.dd*	6.48 *m*	6.48 *br.dd*	6.69 *br.dd*	5.81 *br.dd*	5.18/5.51 *br.dd*	5.14 *m*	5.22/5.59 *br.d*
14a	2.00/2.10 *m*	)	2.00 *m*	~2.00 *m*	2.05 *m*	1.90 *m*	1.70/1.70 *m*	1.80 *m*	1.75/1.75 *m*
14b	2.10/2.25 *m*	)1.80–	2.00 *m*			2.05 *m*			1.95/1.95 *m*
15a	2.05/2.05 *m*	)2.00 *m*	1.95 *m*	1.95 *m*	1.90 *m*	1.90 *m*	1.70/1.70 *m*	1.70 *m*	1.75/1.75 *m*
15b		)	1.75 *m*						
16a	3.65/3.30 *m*	3.65 *m*	3.37 *m*	3.31 *m*	3.45 *m*	3.20 *m*	3.13/3.30 *m*	3.16 *m*	3.50/3.50 *m*
16b	3.65/3.88 *m*		3.55 *m*	3.55 *m*		3.65 *m*	3.63/3.30 *m*	3.63 *m*	
19	1.83/2.38 *sept*	1.98 *sept*	1.75 *m*	1.89 *sept*	2.28 *sept*	2.55 *sept*	2.05/2.08 *sept*	1.87 *m*	2.06/2.43 *sept*
20	0.76/1.16 *d*[d]	0.81 *d*[d]	a: 1.30 *m*	0.92 *d*[d]	1.12 *d*[d]	1.28 *d*	0.73/0.90 *d*	a: 1.25 *m*	0.97/1.19 *d*[d]
			b: 1.50 *m*					b: 1.40 *m*	
21	0.82/1.18 *d*[d]	0.83 *d*[d]	0.80 *t*	0.97 *d*[d]	1.16 *d*[d]	1.30 *d*	0.96/0.99 *d*	0.77 *t*	1.02/1.23 *d*[d]
22	–	–	0.83 *d*	–	–	–	–	0.68 *d*	–
2′6′	7.62/7.62 *br.d*	7.50 *br.d*	7.38 *br.d*	7.01 *d*	7.09 *d*	6.91 *d*	7.37/7.34 *d*	7.38 *d*	7.36/7.35 *d*
3′5′	6.85/6.85 *br.d*	6.89 *br.d*	6.63 *br.d*	6.61 *d*	6.75 *d*	6.71 *d*	6.73/6.71 *d*	6.74 *d*	6.69/6.69 *d*
2″,6″	7.10/7.10 *m*	6.56 *m*	7.15 *m*	)7.00–	6.83 *m*	6.96 *m*	7.59/7.59 *d*	7.58 *m*	7.60/7.60 *d*
3″,5″	6.85/6.90 *m*	)7.00–	6.95 *m*	)7.10	)6.95–	)7.11	)7.26/	)7.20–	)7.20–7.30/
4″	7.10/7.10 *m*	)7.10 *m*	6.95 *m*	)*m*	)7.05 *m*	*m*	)7.26 *m*	)7.30 *m*	)7.20–7.30 *m*
6-OCH_3	4.27/4.18 *s*	4.34 *s*	4.02 *s*	3.99 *s*	3.98 *s*	4.00 *s*	3.63/3.73 *s*	3.65 *s*	3.79/3.79 *s*
4′-OCH_3	3.78/3.79 *s*	3.81 *s*	3.69 *s*	3.69 *s*	3.73 *s*	3.74 *s*	3.72/3.73 *s*	3.73 *s*	3.72/3.74 *s*
OCH_2O a	6.01/5.97 *d*	6.01 *d*	5.86 *s*	5.87 *d*	5.91 *d*	5.93 *d*	5.93/5.95 *d*	5.93 *d*	5.98/5.97 *d*
OCH_2O b	5.91/5.91 *d*	5.89 *d*	5.86 *s*	5.85 *d*	5.89 *d*	5.91 *d*	5.88/5.93 *d*	5.89 *d*	5.93/5.94 *d*
5-OH	5.82/5.56 *s*	?	5.82 *s*	5.67 *s*	?	2.85 *s*	–	–	–
10[c]	5.60/5.58 *d*	~2.00 *d*	3.66 *s*	2.41 *s*	–	–	3.21/3.16 *s*	3.21 *s*	3.14/3.16 *s*
NH	5.33/6.13 *br.d*	5.39 *br.d*	5.18 *br.d*	5.11 *br.d*	5.74 *br.d*	6.45 *br.d*	5.64/5.43 *br.d*	5.69 *br.d*	5.12/? *br.d*

[a] Coupling constants. **3**: J(3, 4) = 6.0 Hz, J(10, 10-OH) = ca 11 Hz, J(13, NH) = ca 7 Hz, J(19, 20) = J(19, 21) = 6.9 Hz, J(2′, 3′) = J(5′, 6′) = 9.0 Hz, J_{gem}(OCH_2O) = 1.4 Hz; **4**: J(3, 4) = 5.1 Hz, J(10, 10-OH) = ca 5.3 Hz, J(13, NH) = ca 8 Hz, J(19, 20) = J(19, 21) = 6.8 Hz, J(2′, 3′) = J(5′, 6′) = 8.9 Hz, J_{gem}(OCH_2O) = 1.6 Hz; **5**: J(3, 4) = 10.0 Hz, J(13, NH) = 7.5 Hz, J(19, 22) = 6.8 Hz, J(20, 21) = 7.0 Hz, J(2′, 3′) = J(5′, 6′) = 9.1 Hz; **6**: J(3, 4) = 9.6 Hz, J(13, 14a) = 5.2 Hz, J(13, 14b) < 1 Hz, J(13, NH) = 7.5 Hz, J(19, 20) = J(19, 21) = 7.0 Hz, J(2′, 3′) = J(5′, 6′) = 8.9 Hz, J_{gem}(OCH_2O) = 1.4 Hz; **7**: J(3, 4) = 13.5 Hz, J(13, 14a) = 6.5 Hz, J(13, 14b) < 1 Hz, J(13, NH) = ~9 Hz, J(19, 20) = J(19, 21) = 6.8 Hz, J(2′, 3′) = J(5′, 6′) = 8.9 Hz, J_{gem}(OCH_2O) = 1.5 Hz; **8**: J(3, 4) = 13.0 Hz, J(13, 14a) = 6.4 Hz, J(13, 14b) < 1 Hz, J(13, NH) = 8.3 Hz, J(19, 20) = J(19, 21) = 6.9 Hz, J(2′, 3′) = J(5′, 6′) = 8.9 Hz, J_{gem}(OCH_2O) = 1.4 Hz; **9**: J(3,4) = 10.1 Hz (**A**)/9.6 Hz (**B**), J(13, 14a) = 3.7 Hz, J(13, 14b) < 1 Hz, J(13, NH) = 6.9 Hz, J(19, 20) = J(19, 21) = 6.8 Hz, J(2′, 3′) = J(5′, 6′) = 9.1 Hz, J(2″, 3″) = J(5″, 6″) = 8.0 Hz, J_{gem}(OCH_2O) = 1.4 Hz; **10**: J(3, 4) = 10.0 Hz, J(13, NH) = 6.6 Hz, J(19, 22) = 6.8 Hz, J(20, 21) = 7.5 Hz, J(2′, 3′) = J(5′, 6′) = 8.9 Hz, J_{gem}(OCH_2O) = 1.4 Hz; **11**: J(3, 4) = 9.4 Hz (**A**)/9.1 Hz (**B**), J(13, NH) = 5.5 Hz, J(19, 20) = J(19, 21) = 6.9 Hz, J(2′, 3′) = J(5′, 6′) = 8.9 Hz, J_{gem}(OCH_2O) = 1.4 Hz.

[b] **3**, **9** and **11** were obtained as diastereomeric mixtures with 13 *S* or 13*R* configuration; ratios **3A**:**3B** = 5:3, **9A**:**9B** = 2:1, and **11A**:**11B** = 5:4.

[c] OH for **3**–**5**, $OCOCH_3$ for **6**, and $COOCH_3$ for **9**–**11**.

[d] Interchangeable within the columns.

eton to a putrescine derived 2-aminopyrrolidine moiety which is again linked either to 2-methylpropanoic acid (in **3**, **4** and **6**) or to 2-methylbutanoic acid (in **5**). The latter corresponds to the well-known bisamide odorine, the former to piriferine, both containing a cinnamic acid part instead of the flavagline type acid and have frequently been found in *Aglaia* species (Brader et al., 1998; Dumontet et al., 1996; Saifah et al., 1993; Purushothaman, Sarada, Connolly & Akinniyi, 1979). As far as the flavagline skeleton is concerned, compounds **3**–**6** are characterized by a cyclopenta[*bc*]benzopyran (=2,5-methano-1-benzoxepin) skeleton, which could be derived from ^{1}H and ^{13}C NMR data including extensive use of 2-D techniques, especially NOESY and HMBC (see discussion below). This type of flavagline nucleus has recently been described for aglains A, B, and C from *A. argentea* Blume (Dumontet et al., 1996). However, the derivatives described therein possess no methylenedioxy group at ring A, and they are all linked to an odorine-like bisamide moiety. In our case the piriferine-like bisamide structures are highly preponderant.

The cyclopenta[*bc*]benzopyran and the cyclopenta[*b*]benzofuran structures are closely related: the former may be converted to the latter by opening bond 5–5a and closing 5a–10. This may occur via a carbenium ion rearrangement after a loss of OH^- at position 10. Dreiding models show, that during a rearrangement like that, the configurations at the quaternary C-2 (=position 4 in the numbering of the

Table 2
^{13}C-NMR data for compounds **3**–**11**

	3A/B[a]	**4**	**5**	**6**[b]	**7**	**8**	**9A/B**[a,c]	**10**[c]	**11A/B**[a,c]
2	89.6/89.7 *s*	85.9 *s*	88.6 *s*	87.8 *s*	99.4 *s*	99.7 *s*	90.6/[d] *s*	90.5 *s*	[d]
3	56.2/55.0 *d*	55.4 *d*	57.3 *d*	56.7 *d*	55.6 *d*	55.6 *d*	51.2/50.4 *d*	51.2 *s*	50.3/49.9 *d*
4	61.3/62.1 *d*	56.7 *d*	63.0 *d*	63.4 *d*[e]	51.2 *d*	52.8 *d*	63.7/65.3 *d*	63.9 *d*	64.7/65.3 *d*
5	80.1/79.8 *s*	80.7 *s*	82.2 *s*	81.9 *s*	88.9 *s*	89.2 *s*	192.1/[d] *s*	192.2 *s*	191.9/192.4 *s*
5a	112.5/113.1 *s*	[d]	107.3 *s*	107.4 *s*	109.1 *s*	109.1 *s*	117.8/[d] *s*	[d]	[d]
6	139.0/138.7 *s*	[d]	140.1 *s*	140.3 *s*	141.2 *s*	141.3 *s*	141.5/[d] *s*	141.6 *s*	140.6/140.2 *s*
7	130.3/130.5 *s*	[d]	129.6 *s*	130.0 *s*	130.7 *s*	131.0 *s*	134.9/133.5 *s*	134.8 *s*	[d]
8	149.6/149.3 *s*	[d]	149.6 *s*	149.7 *s*	152.8 *s*	153.0 *s*	154.6/153.4 *s*	154.7 *s*	[d]
9	92.8/93.4 *d*	92.5 *d*	93.6 *d*	93.5 *d*	88.9 *d*	89.0 *d*	99.8/98.4 *d*	99.8 *d*	97.9/98.1 *d*
9a	147.2/147.4 *s*	[d]	147.5 *s*	147.1 *s*	154.8 *s*	154.8 *s*	152.3/151.7 *s*	[d]	152.5/151.6 *s*
10	83.2/83.0 *d*	74.1 *d*	79.9 *d*	79.4 *d*	205.6 *s*	207.1 *s*	170.1/[d] *s*	170.3 *s*	[d]
11	173.7/172.4 *s*	169.8 *s*	169.7 *s*	169.0 *s*	164.6 *s*	166.6 *s*	167.3/165.9 *s*	[d]	167.1/166.2 *s*
13	64.1/65.7 *d*	63.5 *d*	63.4 *d*	63.5 *d*[e]	62.7 *d*	63.5 *d*	64.2/65.5 *d*	64.5 *d*	63.4/65.1 *d*
14	34.1/31.1 *t*	34.2 *t*	34.3 *t*	34.3 *t*	33.8 *t*	34.4 *t*	34.2/30.8 *t*	34.2 *t*	33.6/31.7 *t*
15	21.5/23.7 *t*	21.5 *t*	21.2 *t*	21.2 *t*	21.6 *t*	21.8 *t*	21.4/23.5 *t*	21.3 *t*	21.3/23.0 *t*
16	46.4/48.1 *t*	45.9 *t*	46.0 *t*	45.9 *t*	45.7 *t*	46.6 *t*	46.3/46.7 *t*	46.4 *t*	46.0/46.2 *t*
18	175.4/177.6 *s*	175.9 *s*	175.4 *s*	175.0 *s*	175.8 *s*	176.6 *s*	176.3/[d] *s*	175.8 *s*	17.53/[d] *s*
19	34.9/35.7 *d*	35.2 *d*	42.1 *d*	34.9 *d*	35.5 *d*	35.7 *d*	34.5/35.5 *d*	41.7 *d*	35.3/35.5 *d*
20	19.1/19.6 *q*	19.1 *q*	26.4 *t*	19.8 *q*	19.8 *q*[e]	20.0 *q*[e]	17.8/19.25 *q*	27.8 *t*	19.1/19.55 *q*
21	18.9/19.3 *q*	19.0 *q*	11.5 *q*	18.7 *q*	18.9 *q*[e]	19.0 *q*[e]	20.1/19.25 *q*	11.6 *q*	19.53/19.6 *q*
22	–	–	16.5 *q*	–	–	–	–	15.6 *q*	–
1′	129.9/129.7 *s*	[d]	[d]	128.9 *s*	125.8 *s*	125.4 *s*	127.7/[d] *s*	[d]	127.1/[d] *s*
2′, 6′	125.6/125.6 *d*	127.1 *d*[e]	130.3 *d*[e]	129.9 *d*[f]	128.1 *d*	128.4 *d*[e]	129.6/129.4[e] *d*	129.6 *d*	129.6/129.6 *d*
3′, 5′	113.1/113.0 *d*	113.3 *d*	112.9 *d*	113.1 *d*	113.3 *d*	113.3 *d*	113.7/113.7 *d*	113.7 *d*	113.7/113.7 *d*
4′	158.9/159.0 *s*	158.9 *s*	158.6 *s*	158.8 *s*	158.8 *s*	159.0 *s*	159.6/[d] *s*	159.7 *s*	
1″	137.2/137.4 *s*	[d]	141.8 *s*	141.5 *s*	136.5 *s*	135.8 *s*	139.2/140.1 *s*	139.2 *s*	
2″, 6″	128.0/126.8 *d*	129.3 *d*	130.2 *d*[e]	129.7 *d*[f]	128.3 *d*	128.1 *d*[e]	129.6/129.5[e] *d*	129.6 *d*[e]	129.6/129.6 *d*
3″, 5″	129.3/129.2 *d*	127.6 *d*[e]	127.9 *d*	128.1 *d*[f]	127.6 *d*	127.8 *d*[e]	128.4/128.2 *d*	128.4 *d*[e]	128.2/128.4 *d*
4″	126.78/126.84 *d*	126.8 *d*	125.8 *d*	126.2 *d*	126.7 *d*	127.1 *d*	127.7/127.5 *d*	127.7 *d*	127.4/127.6 *d*
6-OCH_3	60.6/60.6 *q*	60.6 *q*	60.2 *q*	60.2 *q*	59.7 *q*	59.7 *q*	61.7/61.1 *q*	61.6 *q*	60.8/60.9 *q*
4′-OCH_3	55.2/55.2 *q*	55.2 *q*	55.1 *q*	55.0 *q*	55.1 *q*	55.1 *q*	55.2/55.1 *q*	55.2 *q*	55.08/55.11 *q*
OCH_2O	101.2/101.2 *t*	101.0 *t*	101.0 *t*	101.0 *t*	101.3 *t*	101.4 *t*	102.0/101.9 *t*	102.0 *t*	101.8/101.9 *t*

[a] **3A** and **3B** are diastereomeric at C-13; see footnote [b] of Table 1.
[b] 10-$OCOCH_3$ **6**: 170.5 *s* (CO), 21.3 *q* (CH_3).
[c] 10-(CO)-OCH_3 **9**: 52.14/52.10 *q*; **10** 52.15 *q*; **11**: 52.15/52.05 *q*.
[d] Non-detectable quaternary carbon.
[e,f] Interchangeable within the columns.

cyclopenta[*b*]benzofurans) remains unchanged in both structures. This is important, because the absolute configurations of the cyclopenta[*b*]benzofuran flavaglines are already known from the enantioselective synthesis of rocaglamide (Trost, Greenspan, Yang & Saulnier, 1990). All other related derivatives were correlated either by CD spectra showing a strong negative Cotton effect for the long wave UV band (Nugroho et al., 1997a,b), or simply via the $[\alpha]_D$ value for closely related derivatives. If the cyclopenta[*bc*]benzopyran system is correlated to the topology of the known cyclopenta[*b*]benzofuran system, the C-10 bridge between C-2 and C-5 of the oxa-cycloheptene ring has to point upwards in the usually chosen presentation of the formulas. As a consequence, the 2-(*p*-methoxyphenyl) substituent and the 5-OH group have to point down (α-positions). The substituents at C-3 and C-4 with variable α or β positions can now be correlated relatively to the absolute configurations of the bridgehead substituents. In other words, from NMR data the relative configurations can be derived, the absolute configurations (implied in the structural formulas) are derived from the known absolute configurations of the related cyclopenta[*b*]benzofuran flavaglines.

The assignments of the ^{1}H and ^{13}C NMR resonances were determined by 2-D correlations (C,H-COSY, H,H-COSY HMBC, and NOESY) and are listed in Tables 1 and 2, respectively. The relative configurations of the variable positions at C-3 (phenyl ring), C-4 (amide link), C-10 (OH or OAc), and the diastereomerically labile C-13 (between two N atoms) were determined by NOESY. In the case of compound **3** (mixture **A**:**B** = 5:3), NOESY cross peaks 3-H/2′6′-H, 2″6″-H, and 10-OH indicate the position 3β for the proton at C-3 and an *endo* arrangement of 10-OH relative to the 3β-H. Cross peaks 4-H/2″6″-H, 13-H, 5-OH, NH for **3A** are compatible with a 4α-H and a 13 *S* configuration at C-13. The special orientation of

the bisamide side chain can be used to discriminate the 13 *S* and 13*R* configurations by NOESY cross peaks: only a 13 *S* configuration allows the 13-H/4-H and the 20,21-H_3/2″6″-H cross peaks (Dumontet et al., 1996). The main component **3A** showed cross peaks 13-H/4-H, 14-H, 15-H, NH and 20,21-H_3/19-H, NH, 2″6″-H. For the minor component **3B** no cross peaks 13-H/4-H and 20,21-H_3/2″6″-H were observed (all others are the same for both). **3A**, **3B** is therefore an epimeric mixture with 13 *S* (**3A**) and 13*R* (**3B**) configurations. It should be noted that epimerization at the position between the two nitrogen atoms (N analogon of acetals) seems to occur easily (compare Brader et al., 1998; Purushothaman et al., 1979).

Compound **4** differs from **3** by a change of the configuration of the OH group at the C-10 bridge. This can be concluded from the cross peak 3-H/10-H proving unambiguously a 3β-H with an *endo* relation to 10-H (or *exo* to 10-OH). A strong 4-H/2″6″-H cross peak indicates 13 *S* configuration (all other NOESY cross peaks are virtually the same for **3** and **4**). In contrast to compounds **3**, **4**, and **6**, compound **5** is characterized by an odorine type side chain and differs from aglain C (Dumontet et al., 1996) only by the presence of a methylenedioxy group in ring A. All other ^{1}H and ^{13}C data of compound **5** and aglain C (including HMBC and NOESY) are very similar. The cross peaks H-3/2′6′-H, 2″6″-H and H-4/13-H, 2″6″-H, 10-OH prove the structure as depicted in the formula scheme with a 3α-H and 4β-H substitution pattern. The 13 *S* configuration follows again from the NOESY relationship H-4/13-H and is additionally indicated by the NOESY cross peaks 21-H_3/2″6″-H, 3″/5″-H (compare Dumontet et al., 1996). The change of the relative configurations at C-3 and C-4 is also reflected in the coupling constant *J*(3,4) which amounts to 6.0 Hz in **3** and 5.1 Hz in **4** (both 3β, 4α), but to a value of 10.0 Hz in **5** (3α, 4β). A coupling constant *J*(3,4) = 9.6 Hz for acetate **6** implies also 3α-H and 4β-H configuration which follows also from the expected NOESY cross peaks. Of special interest are here the NOESY relationships between the 10-acetate group and the unsubstituted phenyl ring at C-3. These cross peaks, 10-acetyl/2″6″-H and 2″6″-H/3-H, 4-H, 10-acetyl, 20-H_3, 21-H_3 prove the *endo* position of 10-OCOCH_3 and the 13 *S* configuration which allows a close approach of the 20,21-dimethyl groups to the unsubstituted phenyl ring (the close 4-H and 13-H protons show also the expected mutual cross peak 4-H/13-H).

In the ^{1}H NMR spectra of compounds **7** and **8** the bridge methine protons C-10 (H, OH) were missing. Since an additional keto resonance appeared in the ^{13}C NMR spectra, 10-OH has obviously been oxidized to a keto carbonyl function. HMBC and NOESY (e.g. 3-H/13-H, 2′6′-H, 2″6″-H and 4-H/13-H, 2″6″-H) allowed the assignments of the resonances as listed in Tables 1 and 2 and the determination of configuration 13 *S* for the side chain. However, the relative configurations at C-3 and C-4 cannot be derived from these data. The reason is the lack of protons at the C-10 bridge which could show a NOESY correlation with β orientated protons at either C-3 or C-4. This problem could be solved elegantly with the use of Eu(fod)$_3$ as shift reagent. Although the molecules **7** and **8** are polyfunctional, the coordination takes place almost exclusively at the 10-keto function. Ethers show generally very small coordination constants, and also amides and tertiary alcohols show usually less coordination to lanthanide shift reagents than ketones (Hofer, 1976; Shapiro et al., 1973). Assuming that the lanthanide induced shifts (LIS) for 3-H and 4-H are caused mainly by coordination at the C-10 keto carbonyl, the LIS results allowed a straightforward interpretation. In compound **7** the proton 4-H showed a larger LIS value than 3-H (4-H 0.43 ppm, 3-H 0.27 ppm), in compound **8** the result was opposite (4-H 0.34 ppm, 3-H 0.67 ppm). This is only compatible with a 3α-H, 4β-H configuration in **7** and a 3β-H, 4α-H configuration in **8** because the larger LIS value is only possible for the β position pointing towards the coordinating keto carbonyl (which is also β). In this context the assignment of 3-H and 4-H is the crucial point. However, this follows clearly from the NOESY cross peaks of 3-H with protons of both aromatic rings Ar′ and Ar″ at C-2 and C-3 and the usual cross peak of 4-H with the side chain proton 13-H (compare also compounds **3**–**6**). Concerning the LIS values of compound **8** one further fact deserves some comment. For some sterical reason(s), considerable coordination takes place also at the amide carbonyl at position 15. This is indicated by high LIS values for 13-H, 19-H, and for both terminal methyl groups (20- and 21-CH_3) of compound **8** (see Section 3 for details). However, this second independent coordination size is far away from the decisive protons 3-H and 4-H and should have only little influence on the LIS values of protons close to the main complexation at the 10 keto carbonyl.

The ^{1}H and ^{13}C NMR data of compounds **9**–**11** showed many characteristic features of the flavaglines **1**–**8**, e.g. the methylenedioxy-methoxy substituted aromatic ring A, a *para*-methoxysubstituted aromatic ring B (Ar′), a simple phenyl ring C (Ar″), and a methine pair (3-H, 4-H), coupling with each other. Compounds **9** and **11** showed additionally the characteristic signals of a piriferin type moiety, whereas **10** was characterized by an analogous odorine derived bisamide side chain. Compared to ketones **7** and **8**, the keto carbonyl function was missing, however, new resonances typical for a —COOCH_3 group appeared in the ^{13}C and ^{1}H NMR spectra (ester carbonyl and ester OCH_3 group; compare Tables 1 and 2 and IR-data). This suggested a structure which could be derived from the cyclopen-

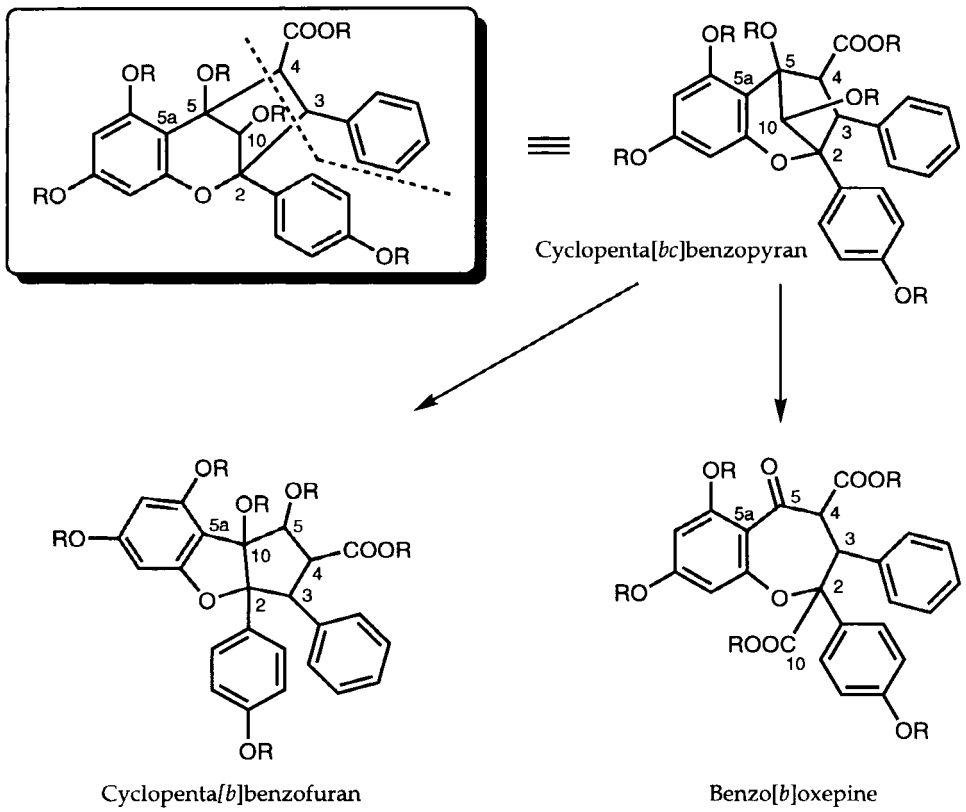


Fig. 1. Supposed biosynthetic connections between flavagline skeletons.

ta[*bc*]benzopyran type flavaglines by an oxidative opening of the 2,5-methano bridge. Two similar derivatives, forbaglin A and B, which have already recently been reported (Dumontet et al., 1996), differ only by the configuration at C-13 (epimers 13*R* and 13 *S*, respectively). In our case, compounds **9** and **11** were obtained as epimeric mixtures with a preponderant content of the 13 *S* epimer. On the basis of the absolute configurations known from the related cyclopenta[*b*]benzofuran flavaglines, and the relative configurations derived from X-ray crystallography, the absolute configuration at C-13 was determined as 13*R* for forbaglin A (Dumontet et al., 1996). One characteristic feature of the 13*R* epimer was the close approach of 19-H_3 of the odorine rest to the unsubstituted phenyl ring Ar″, showing up in the NOESY spectrum of forbaglin A (Dumontet et al., 1996). In the case of the minor components of our piriferine derived compounds **9B** and **11B** the same effect was observed for 19-/20-H_3 of the side chain and the Ar″ ring (compare NOESY data, Section 3). However, this was only the case for the minor components **B** of the epimeric mixtures **9** and **11**; the preponderant epimers **9A** and **11A** (13 *S*) did not show these cross peaks. The assignments of 13 *S* to epimers **A** and 13*R* to epimers **B** are additionally supported by some characteristic ^{13}C NMR differences between both forms. Especially the ^{13}C NMR shifts of C-14 and C-15 for all oxepine flavaglines seem to be well suited for the discrimination of 13 *S* and 13*R* configuration: C-14: 33.6–34.4 (13 *S*), 30.8–31.7 (13*R*); C-15: 21.2–21.8 (13 *S*), 23.0–23.7 (13*R*), compare Table 2, compounds **3**–**11**. With the exception of Ring A (and the piriferin derived side chain of **9**), the ^{13}C NMR resonances for the 13 *S* configurated compounds **9A**, **10** and forbaglin B were virtually the same (this is also true for the 13*R* epimers **9B** and forbaglin A). The relative configurations at positions 3 and 4 are therefore identical for **9**, **10**, and the forbaglins: 3α-H and 4β-H (Dumontet et al., 1996).

Compound **11** (13 *S*/13*R* pair **A**/**B**) proved to be isomeric to **9**(**A**/**B**). This was indicated by identical molecular masses and EI mass spectra without any significant differences between **9** and **11**. The ^{13}C NMR data of compounds **11A**/**B** and **9A**/**B** differed only slightly but significantly. In the ^{1}H NMR spectrum especially the chemical shifts of protons H-3 and H-4 showed different chemical shift values allowing the conclusion that the substitution of the phenyl group Ar″ and the bisamide side chain may be different in **9** and **11**. In principle, several isomeric relationships are possible at C-3 and C-4: either epimers by changing the configuration of only one substituent, diastereomers by changing both configurations (3β-H and 4α-H

Table 3
LC_{50} and EC_{50} values of flavaglines and azadirachtin against neonate larvae of *Spodoptera littoralis*[a]

Compound	Survival rate LC_{50} (95% FL[a]) µg/g fr. wt.	Growth inhibition EC_{50} (95% FL[b]) µg/g fr. wt.
Aglaroxin-A (**1**)	3.4 (2.2–5.2)	0.21 (0.19–0.22)
Pannellin (**2**)	2.1 (1.2–4.3)	0.24 (0.20–0.29)
Thapsakin-A 10-O-acetate (**6**)	> 50	> 50
Thapoxepine-A (**9**)	> 50	> 50
Rocaglamide	1.1 (0.8–2.6)	0.14 (0.12–0.17)
Aglafolin (= methyl rocaglate)	2.9 (2.6–3.1)	0.25 (0.19–0.31)
Rocaglaol	> 20	3.40 (2.08–5.95)
Azadirachtin	6.1 (4.1–11.0)	0.11 (0.05–0.17)

[a] Neonate larvae of *S. littoralis* ($n = 20$) were released on bean based artificial diet (3.8 g) spiked with various concentrations of the test compounds. After 5 days, survival rate and growth inhibition were determined in triplicate and compared to controls. LC_{50} and EC_{50} were determined by probit-log analysis.
[b] Fiducial limits.

like in compounds **3**, **4** and **8**), or constitutional isomers by exchange of the substituents [3-bisamide, 4-phenyl, as described for aglaforbesin A and B (Dumontet et al., 1996)]. Unfortunately, no direct evidence by NOE or HMBC was possible to prove either one of the isomeric structures in a straightforward and independent manner. Due to the flexibility of the unbridged and therefore conformationally flexible 7-ring (pseudorotation), the LIS method failed as well. However, in the light of all experimentally derived regularities of the NMR data of numerous flavaglines, only a change of the configurations at C-3 and C-4, resulting in the 3β-H and 4α-H diastereomer is compatible with the data of compound **11**. In all flavaglines known so far (including the cyclopenta[*b*]benzofurans), the benzylic protons are found at higher chemical shift values, than the vicinal methine proton at the carbon atom carrying the amide side chain. In the case of compound **11A**/**B** this is the proton at δ 5.30/5.28. In all cases measured for the benzoxepine type flavaglines with a phenyl ring in position 3, the benzylic (low field) methine protons show a NOESY relationship to both aromatic rings B and C (Ar′ and Ar″). These NOESY cross peaks are not possible if the unsubstituted phenyl ring C is positioned at C-4 [compare the 4-phenyl-3-bisamide substituted aglaforbesins of (Dumontet et al., 1996)]. In the case of **11A**/**B** strong NOESY cross peaks of the benzylic 3-H with 2′6′ and 2″6″ proves position 3 for the phenyl substituent and consequently position 4 for the bisamide substituent. The vicinal coupling constants $J(3,4)$ which are only slightly different in **9** and **11** are characteristic for a transoid arrangement of protons 3-H and 4-H. The only isomeric possibility left is therefore a diastereomeric structure with configurations 3β-H, 4α-H for **11** compared to 3α-H, 4β-H for **9**. Further support that **11** is not a constitutional isomer of **9** is due to the 10-OCH_3/2″6″ cross peak for **9** and **11**, which indicates a vicinal relationship between the —$COOCH_3$ and the phenyl group at C-3. Finally, it should be pointed out that for all benzoxepine flavaglines presented in this paper, the 3α-H, 4β-H derivatives were isolated in larger amounts than the diastereomeric 3β-H, 4α-H products (compounds **5** and **6** versus **3** and **4**, and the diastereomeric pairs **7**/**8** and **9**/**11**).

The co-occurrence of cyclopenta[*b*]benzofuran flavaglines (**1**, **2**) with similarly substituted benzo[*bc*]pyran (**3**–**8**) and benzo[*b*]oxepine derivatives (**9**–**11**) in *A. edulis* suggests a common biogenetic origin. This is also supported by a previous report on *A. argentea* and *A. forbesii*, where flavaglines of all three structural types showed a common substitution pattern of the aromatic rings only deviating from *A. edulis* by a dimethoxylated A-ring. In the proposed biosynthetic pathway a flavonoid nucleus has been suggested to be linked with a cinnamic acid moiety to form the cyclopenta[*bc*]benzopyran skeleton (Dumontet et al., 1996). The transformation to the corresponding benzo[*b*]-furan structures can be explained by opening bond 5–5a and closing 10–5a, whereas the benzo[*b*]oxepines can be derived by an oxidative cleavage of the 2,5-methano bridge between 5–10 (Fig. 1).

As already reported previously, the two flavaglines with a benzofuran skeleton, aglaroxin A (**1**) (Ciba-Geigy, 1996) and pannellin (**2**) (Brader et al., 1998), exhibited significant insect toxicity against neonate larvae of *S. littoralis*. By contrast, the benzopyran (**6**) and benzoxepine derivatives (**9**) did not show a significant effect (Table 3). The well-known benzofurans rocaglamide (King et al., 1982), aglafolin (= methyl rocaglate) (Ko, Wu, Liou, Huang & Teng, 1992), and rocaglaol (Ishibashi, Satasook, Isman & Towers, 1993) were also isolated from the roots of *A. odorata* Lour., collected in Kho Samet (see Section 3), to enable a direct comparison of LC_{50} and EC_{50} values. In addition, the limonoid azadirachtin, purchased from Roth (Karlsruhe, Germany), was used as a positive control. As shown in Table 3, the survival and growth inhibition rates of all benzofurans are very similar with rocaglamide as the most active, and rocaglaol as the less active derivative. Similar results have also previously been obtained against larvae of *Peridroma saucia* (Ishibashi et al., 1993). Concerning the lethal concentration (LC_{50}) against *S. littoralis* it is interesting to note, that with the exception of rocaglaol all benzofurans showed higher insect toxicities than azadirachtin (Table 3).

According to Pannell (1992), *A. edulis* comprises 35 synonyms which cannot conveniently be subdivided into separate species or subspecies on present morphological characters. With respect to the very character-

istic patterns of flavaglines and/or bisamides known so far only from the genus *Aglaia*, taxonomic contributions can also be expected from phytochemical characters. In our case *A. edulis*, collected in southwest Thailand (HG 515, see Section 3), can be clearly distinguished from a previous collection in southeast Thailand (HG 12) (Brader et al., 1998) by the accumulation of flavaglines. A segregation of both provenances is also suggested by different types of indumentum showing pale brown stellate hairs in the former and peltate scales in the latter. However, since intermediate representatives are to be expected which have both hairs and scales (Pannell, 1992), only further collections with flowers and fruits could enable us to come up to a final taxonomic decision.

3. Experimental

3.1. General

NMR: Bruker, AM 400 WB and DPX 250. MS: Finnigan MAT 900 S. IR: Perkin-Elmer 16PC FT-IR. UV: Hewlett-Packard, 8452A Diode Array Spectrophotometer. Optical rotation: Perkin Elmer Polarimeter 241. HPLC: Hewlett-Packard 1090 II, UV diode array detection at 230 nm, column 250 × 4 mm, Hypersil BDS C-18, 5 μm, mobile phase MeOH (gradient 60–100%) in aqueous buffer (0.015 M *o*-phosphoric acid, 0.0015 M tetrabutylammonium hydroxide, pH 3), flow rate 1 ml/min.

3.2. Plant material

The root bark of *A. edulis* was collected near Khao Lan Waterfall, Thap Sakae, Prachuap Khiri Khan (southwest Thailand) 4 July 1998. Voucher specimens (HG 515) are deposited at the Herbarium of the Institute of Botany, University of Vienna (WU).

3.3. Extraction and isolation

Dried root bark, 263 g, was ground and extracted with MeOH at room temp. for 3 days, filtered and concd. The $CHCl_3$ fraction from the aqueous solution was evapd to dryness under reduced pressure (2400 mg) and roughly sepd by column chromatography (Merck silica gel 60, 35 to 70 mesh) with hexane, EtOAc and MeOH. The fractions eluted with pure EtOAc were further sepd by prep. MPLC with 30% (v/v) EtOAc in hexane (400 × 38 mm column, Merck LiChroprep Si 60, 25 to 40 μm, UV detection, 254 nm) followed by prep. TLC (Merck silica gel 60, F_{254}) with 4% MeOH in $CHCl_3$ to yield 24 mg aglaroxin-A (**1**), 3 mg pannellin (**2**), 2 mg isothapsakin-B (**4**), 5 mg homothapsakin-A (**5**), 22 mg thapsakin-A 10-*O*-acetate (**6**), 4 mg thapsakon-A (**7**) and 9 mg thapsakon-B (**8**). Purification by repeated MPLC with 50% (v/v) EtOAc in hexane (400 × 38 mm column, Merck LiChroprep Si 60, 25 to 40 μm, UV detection, 254 nm) afforded 41 mg thapoxepine-A (**9**), 7 mg homothapoxepine-A (**10**), 5 mg thapoxepine-B (**11**) as well as impure thapsakin-B (**3**), which were finally purified by prep. TLC (mobile phase: CH_2Cl_2–Et_2O–MeOH, 70:27:3) to amount 6 mg pure material.

3.3.1. Aglaroxin A (Ciba-Geigy, 1996) (−)-(1R,2R,3 S,3aR)-16,7,8,8a-Tetrahydro-8,8a-dihydroxy-9-methoxy-5a-(4-methoxyphenyl)-6-phenyl-5aH-cyclopenta[4,5]furo[2,3-f]-1,3-benzodioxole-7-N,N-dimethyl amide (**1**)

$[\alpha]_D^{20}$ −81° (*c* 0.4, $CHCl_3$). ^{1}H NMR ($CDCl_3$) $\delta = 7.09$ (*d*, 2H, J = 9.0 Hz, 2′- and 6′-H), 6.95–7.05 (*m*, 3H, 3″-, 4″-, and 5″-H), 6.86 (*m*, 2H, 2″- and 6″-H), 6.67 (*d*, 2H, J = 9.0 Hz, 3′- and 5′-H), 6.31 (*s*, 1H, 5-H), 5.89 (*d*, 1H, J = 1.4 Hz, OCH_2O), 5.88 (*d*, 1H, J = 1.4 Hz, OCH_2O), 4.84 (*d*, 1H, J = 6.2 Hz, 1-H), 4.56 (*d*, 1H, J = 13.5 Hz, 3-H), 4.08 (*dd*, 1H, J = 13.5 and 6.2 Hz, 2-H), 4.08 (*s*, 3H, 8-OCH_3), 3.70 (*s*, 3H, 4′-OCH_3), 3.31 (*s*, 3H, N-CH_3), 2.94 (*s*, 3H, N-CH_3). ^{13}C NMR ($CDCl_3$) $\delta = 169.9$ (C═O), 158.6 *s* (C-4′), 154.5 *s* (C-4), 152.0 *s* (C-6), 139.9 *s* (C-8), 137.5 *s* (C-1″), 130.1 *s* (C-7), 128.8 *d* (C-2′/6′), 127.8 and 127.7 (2 × *s*, C-2″/6″ and C-3″/5″), 127.2 *s* (C-1′), 126.3 *d* (C-4″), 112.7 *d* (C-3′/5′), 110.1 *s* (C-8a), 101.5 *s* (C-3a), 101.1 *t* (OCH_2O), 94.6 *s* (C-8b), 88.2 *d* (C-5), 78.5 *d* (C-1), 59.8 *q* (8-OCH_3), 56.3 *d* (C-3), 55.1 *q* (4′-OCH_3), 47.3 *d* (C-2), 37.1 *q* (N-CH_3), 35.8 *q* (N-CH_3). Assignments by comparison with data from Brader et al. (1998) (e.g. pannellin) and CH-COSY. EI-MS (70 eV, 200°C) *m/z*: 519 (7%) M^+, 501 (4), 456 (4), 404 (6), 327 (7), 314 (8), 205 (100), 176 (21), 149 (14), 145 (24), 131 (14), 105 (16), 95 (21), 81 (29), 69 (23), 57 (69).

3.3.2. Thapsakin B (+)-(2R,3R,4 S,5R,10 S,2′RS)-1-[2,3,4,5-Tetrahydro-5,10-dihydroxy-2-(4-methoxyphenyl)-6-methoxy-7,8-methylenedioxy-3-phenyl-2,5-methano-1-benzoxepin-4-carbonyl]-2-(2-methylpropanoylamino)-pyrrolidine (**3**)

Epimeric mixture of (2′*S*)-**3A**: (2′*R*)-**3B** = 5:3. $[\alpha]_D^{20}$ = +24° (*c* = 0.4, $CHCl_3$). UV λ^{MeOH} nm (log ϵ): 252 (*sh*, 3.70), 271 (*sh*, 3.51), 282 (*sh*, 3.54), 298 (3.68). IR ν^{CHCl_3} cm^{-1}: 3434 *w*, 2954 *w*, 2938 *w*, 2835 *w*, 1670 *m*, 1626 *s*, 1606 *s*, 1514 *m*, 1498 *m*, 1476 *s*, 1462 *s*, 1436 *m*, 1360 *w*, 1322 *w*, 1254 *w*, 1178 *s*, 1134 *m*, 1074 *m*, 1060 *m*, 1010 *w*, 966 *w*, 941 *w*. ^{1}H and ^{13}C NMR see Tables 1 and 2. HMBC: 3-H (2 *w*, 4, 11, 1″, 3″5″), 4-H (3 *w*, 5, 5a, 10, 11, 1″), 9-H (5a, 9a, 7, 8), 19-H (20, 21), 20 + 21-H_3 (18), 2′6′-H (2, 4′), 3′5′-H (1′, 4′ *w*), 3″5″-H (4″), 2″6″ + 4″-H (1″, 3″5″), 6-OCH_3 (6), 4-OCH_3 (4′), 5-OH (5, 5a, 10), 10-OH (5, 10).

NOESY: 3-H (2′6′, 3″5″, 10-OH), 4-H (2″6″, 13, 5-OH, NH; for **3B** no cross peak to 13 and to NH), 13-H (4, 14, 15, NH; for **3B** no cross peak to 4), 20 + 21-H_3 (19, NH, 2″6″; for **3B** no cross peak to 2″6″), NH (4, 13, 19; for **3B** no cross peak to 4). EI-MS (70 eV, 210°C) *m/z*: 630 (1%) M^+, 613 (2), 584 (3), 456 (100), 417 (14), 327 (45), 200 (31), 131 (48), 70 (85); FD-MS *m/z*: 630 ($C_{35}H_{38}N_2O_9$, M_{calc} = 630.2577).

3.3.3. Isothapsakin B (−)-(2R,3R,4 S,5R,10R,2′S)-1-[2,3,4,5-Tetrahydro-5,10-dihydroxy-2-(4-methoxyphenyl)-6-methoxy-7,8-methylenedioxy-3-phenyl-2,5-methano-1-benzoxepin-4-carbonyl]-2-(2-methylpropanoylamino)-pyrrolidine (**4**)

$[\alpha]_D^{20} = -51°$ (*c* = 0.2, $CHCl_3$). UV λ^{MeOH} nm (log ϵ): 251 (*sh*, 3.68), 272 (*sh*, 3.45), 282 (*sh*, 3.53), 298 (3.64). IR ν^{CHCl_3} cm^{-1}: 3557 *w*, 3485 *w*, 3432 *w*, 2953 *m*, 2930 *m*, 2853 *w*, 1640 *s*, 1628 *s*, 1514 *s*, 1498 *m*, 1478 *s*, 1462 *s*, 1426 *m*, 1356 *w*, 1322 *w*, 1252 *m*, 1180 *s*, 1168 *m*, 1130 *s*, 1092 *m*, 1064 *m*, 1008 *w*, 938 *w*, 928 *w*, 834 *w*. ^1H and ^{13}C NMR see Tables 1 and 2. NOESY: 3-H (4, 10-H, 2′6′, 2″6″), 4-H (3, 13, 2″6″), 10-H (3, 2′6′), 19-H (20, 21), 2′6′-H (3, 10-H, 3′5′), 3′5′-H (2′6′, 4′-OCH_3), 2″6″-H (3, 4, 3″-5″). EI-MS (70 eV, 210°C) *m/z*: 630 (22%) M^+, 456 (75), 343 (34), 327 (100), 287 (23), 200 (65), 131 (74), 70 (56); FD-MS *m/z*: 630 ($C_{35}H_{38}N_2O_9$, M_{calc} = 630.2577); HR-MS: M_{exp} = 630.2579.

3.3.4. Homothapsakin A (−)-(2R,3 S,4R,5R,10 S,2′S)-1-[2,3,4,5-Tetrahydro-5,10-dihydroxy-2-(4-methoxyphenyl)-6-methoxy-7,8-methylenedioxy-3-phenyl-2,5-methano-1-benzoxepin-4-carbonyl]-2-(2-methylbutanoylamino)-pyrrolidine (**5**)

$[\alpha]_D^{20} = -135°$ (*c* = 0.3, $CHCl_3$). UV λ^{MeOH} nm (log ϵ): 251 (*sh*, 3.76), 272 (*sh*, 3.51), 282 (*sh*, 3.58), 298 (3.69). IR ν^{CHCl_3} cm^{-1}: 3555 *w*, 3485 *w*, 3430 *w*, 2957 *m*, 2932 *m*, 1646 *s*, 1624 *s*, 1612 *m*, 1516 *m*, 1498 *m*, 1476 *s*, 1464 *s*, 1436 *m*, 1420 *m*, 1356 *w*, 1322 *w*, 1250 *m*, 1182 *m*, 1168 *w*, 1128 *m*, 1080 *m*, 1060 *s*, 986 *w*, 956 *w*. ^1H and ^{13}C NMR see Tables 1 and 2. HMBC: 3-H (2, 4 *w*, 11, 1″, 2″6″), 4-H (3 *w*, 5, 5a, 11, 1″), 9-H (5a, 9a, 7, 8), 10-H (3, 4), 19-H (18, 22), 20-H (18), 21 + 22-H_3 (19, 20), 2′6′-H (2, 4′), 3′5′-H (2′6′, 4′), 2″6″-H (4″), OCH_2O (7, 8), 6-OCH_3 (6), 4′-OCH_3 (4′), 5-OH (5, 5a, 10). NOESY: 3-H (2′6′, 2″6″), 4-H (13, 2″6″, 10-OH), 10-H (2′6′, 10-OH), 13-H (4, 14), 21 + 22-H_3 (NH, 2″6″), 2′6′-H (3, 10, 3′5′), 3′5′-H (2′6′, 4′-OCH_3), 2″6″-H (3, 4, 10-OH *w*), 3″5″ + 4″-H (21 or 22 *w*), 6-OCH_3 (16, 5-OH), 4′-OCH_3 (3′5′), 5-OH (13, 6-OCH_3), 10-OH (4, 10), NH (4, 14, 3′5′). EI-MS (70 eV, 210°C) *m/z*: 644 (12%) M^+, 543 (10), 456 (100), 417 (34), 375 (52), 330 (54), 327 (88), 238 (45), 200 (44), 195 (67), 168 (37), 131 (57), 70 (80); FD-MS *m/z*: 644 ($C_{36}H_{40}N_2O_9$, M_{calc} = 644.2734); M_{exp} = 644.2735.

3.3.5. Thapsakin A 10-O-acetate (−)-(2R,3 S,4R,5R,10 S,2′S)-1-[2,3,4,5-Tetrahydro-10-acetyloxy-5-hydroxy-2-(4-methoxyphenyl)-6-methoxy-7,8-methylenedioxy-3-phenyl-2,5-methano-1-benzoxepin-4-carbonyl]-2-(2-methylpropanoylamino)-pyrrolidine (**6**)

Mp. 150 to 152°. $[\alpha]_D^{20} = -24°$ (*c* = 0.5, $CHCl_3$). UV λ^{MeOH} nm (log ϵ): 251 (*sh*, 3.73), 272 (*sh*, 3.48), 282 (*sh*, 3.56), 298 (3.67). IR ν^{CCl_4} cm^{-1}: 3494 *w*, 3436 *w*, 2955 *m*, 2928 *m*, 1758 *m*, 1682 *m*, 1654 *s*, 1628 *m*, 1516 *m*, 1498 *m*, 1476 *s*, 1462 *s*, 1436 *m*, 1420 *m*, 1374 *w*, 1360 *w*, 1254 *m*, 1216 *s*, 1182 *m*, 1130 *m*, 1082 *m*, 1058 *s*, 992 *w*, 964 *w*, 942 *w*, 860 *w*, 700 *m*. ^1H and ^{13}C NMR see Tables 1 and 2. HMBC: 3-H (2, 4, 11, 1″, 2′6′), 4-H (3, 5, 5a, 11, 1″ *w*), 9-H (5a, 8), 10-H (3, 4, $\underline{C}OCH_3$), 19-H (18, 20, 21), 20 + 21-H_3 (18, 19), 2′6′-H (2, 4′, 3′5′), 3′5′-H (1′, 4′), 2″-6″-H (1″-6″), OCH_2O (7, 8), 6-OCH_3 (6), 4′-OCH_3 (4′), 5-OH (5, 5a, 10), 10-OCO$\underline{C}H_3$ ($\underline{C}OCH_3$). NOESY: 3-H (2′6′, 2″6″), 4-H (13, 2″6″, NH), 10-H (2′6′, 5-OH), 13-H (4, 14, 15, 5-OH), 20 + 21-H_3 (NH, 2″-6″), 2′6′-H (3, 10, 3′5′), 3′5′-H (2′6′, 4′-OCH_3), 2″-6″-H (3, 4, CO$\underline{C}H_3$, 20, 21), 6-OCH_3 (5-OH), 4′-OCH_3 (3′5′), 5-OH (4, 10, 13, 6-OCH_3), 10-OCO$\underline{C}H_3$ (2′6′), NH (4, 13, 14 or 15, 3′5′). EI-MS (70 eV, 200°C) *m/z*: 672 (5%) M^+, 498 (10), 456 (36), 417 (18), 327 (20), 220 (19), 205 (56), 149 (17), 131 (18), 111 (19), 97 (31), 85 (42), 71 (52), 69 (47), 59 (58), 57 (100); FD-MS *m/z*: 672 ($C_{37}H_{40}N_2O_{10}$, M_{calc} = 672.2683).

3.3.6. Thapsakon A (+)-(2R,3 S,4R,5R,2′S)-1-[2,3,4,5-Tetrahydro-5-hydroxy-2-(4-methoxyphenyl)-6-methoxy-7,8-methylenedioxy-10-oxo-3-phenyl-2,5-methano-1-benzoxepin-4-carbonyl]-2-(2-methylpropanoylamino)-pyrrolidine (**7**)

$[\alpha]_D^{20} = +23°$ (*c* = 0.6, $CHCl_3$). UV λ^{MeOH} nm (log ϵ): 280 (*sh*, 3.54), 304 (3.65). IR ν^{CCl_4} cm^{-1}: 3493 *w*, 3436 *w*, 2956 *m*, 2926 *m*, 2871 *w*, 1742 *m*, 1654 *m*, 1628 *s*, 1614 *m*, 1514 *s*, 1496 *m*, 1478 *s*, 1454 *s*, 1417 *m*, 1360 *w*, 1314 *w*, 1250 *m*, 1180 *m*, 1158 *s*, 1134 *w*, 1118 *w*, 1084 *m*, 1058 *s*, 994 *w*, 942 *w*, 861 *w*, 702 *m*. ^1H and ^{13}C NMR see Tables 1 and 2. HMBC: 3-H (4, 10, 11, 1″), 4-H (2, 3, 11, 1′, 1″, 2″6″), 9-H (5a, 7, 9a), 20 + 21-H_3 (18, 19), 2′6′-H (2 *w*, 4′), 3′5′-H (1′), 2″6″-H (4″), OCH_2O (7, 8), 6-OCH_3 (6), 4′-OCH_3 (4′). NOESY: 3-H (13, 2′6′, 2″6″), 4-H (13, 2″6″), 13-H (4), 16-H (14), 19-H (20, 21), 20 + 21-H_3 (2″6″, 3″5″), 4′-OCH_3 (3′5′), NH (15, 19). ^1H LIS NMR: 3-H (0.27 ppm), 4-H (0.43), 9-H (0.03), 13-H (~0.22), 14- and 15-H_2 (~0.10), 16a/b-H (~0.15), 19-H (0.09), 20- and 21-H_3 (0.04 and 0.05), 2′6′-H (0.10), 3′5′-H (0.03), 2″6″-H (0.18), 3″5″- and 4″-H (~0.01), OCH_2O a/b (−0.02), 6-OCH_3 (0.03), 4′-OCH_3 (0.003), NH (0.09); the lanthanide induced shifts were determined by adding increasing amounts of Eu(fod)$_3$ (Merck) to a solution of ca 1.5 mg of substrate in 0.5 ml $CDCl_3$ and extrapolation to a ratio substrate:reagent = 1:1; due to the low sub-

strate concentration ([S_0] = 4.8 mM] and the unfavorable complex binding constant, the LIS values are much smaller than for the true 1:1 complex (Hofer, 1976; Armitage, Dunsmore, Hall & Marshall, 1971). EI-MS (70 eV, 220°C) *m/z*: 628 (1%) M^+, 611 (18), 456 (8), 446 (16), 428 (35), 327 (16), 325 (19), 314 (100), 299 (52), 135 (20), 131 (16), 85 (19), 69 (34), 57 (36), 44 (42); FD-MS *m/z*: 628 ($C_{35}H_{36}N_2O_9$, M_{calc} = 628.2421).

3.3.7. Thapsakon B (+)-(2R,3R,4 S,5R,2′S)-1-[2,3,4,5-Tetrahydro-5-hydroxy-2-(4-methoxyphenyl)-6-methoxy-7,8-methylenedioxy-10-oxo-3-phenyl-2,5-methano-1-benzoxepin-4-carbonyl]-2-(2-methylpropanoylamino)-pyrrolidine (**8**)

$[\alpha]_D^{20}$ = +8° (*c* = 0.2, $CHCl_3$). UV λ^{MeOH} nm (log ϵ): 279 (*sh*, 3.60), 304 (3.71). IR ν^{CHCl_3} cm^{-1}: 3538 *m*, 3428 *w*, 2930 *w*, 2834 *w*, 1746 *w*, 1655 *m*, 1648 *m*, 1638 *s*, 1610 *s*, 1602 *s*, 1514 *m*, 1492 *m*, 1452 *s*, 1420 *s*, 1346 *w*, 1326 *w*, 1252 *w*, 1180 *m*, 1162 *m*, 1130 *w*, 1116 *w*, 1086 *w*, 1064 *w*, 994 *w*, 944 *w*. ^1H and ^{13}C NMR see Tables 1 and 2. HMBC: 3-H (4, 10, 11, 1″), 4-H (11, 1′, 1″), 9-H (5a, 7, 9a), 20 + 21-H_3 (18, 19), 2′6′-H (1′, 4′ *w*), 3′5′-H (4′), 6-OCH_3 (6), 4′-OCH_3 (4′), 5-OH (2, 5, 5a). NOESY: 3-H (13, 2′6′, 2″6″), 4-H (13, 2″6″), 13-H (3, 4, 14, 15, 2″6″), 16-H (14, 15), 19 (20, 21), 20 + 21-H_3 (19, NH, 2′6′), 2′6′-H (3, 5-OH, 20, 21), 3′5′-H (4′-OCH_3), 2″6″-H (3, 4, 13), 5-OH (2′6′), NH (4, 15, 19, 20, 21). ^1H LIS NMR: 3-H (0.67 ppm), 4-H (0.34), 9-H (0.08), 13-H (∼1.36), 14-H_2 (∼0.13), 15-H_2 (∼0.03), 16a/b-H (0.32), 19-H (0.77), 20- and 21-H_3 (0.40 and 0.49), 2′6′-H (∼0.26), 3′5′-H (0.09), 2″6″-H (∼0.21), 3″5″- and 4″-H (−0.04), OCH_2O a/b (−0.05), 6-OCH_3 (0.12), 4′-OCH_3 (−0.04), NH (0.86); for comments see thapsakon A). EI-MS (70 eV, 210°C) *m/z*: 628 (2%) M^+, 611 (18), 446 (19), 428 (20), 314 (100), 299 (82), 195 (15), 135 (40), 131 (30), 85 (39), 72 (45), 69 (74), 57 (41); FD-MS *m/z*: 628 ($C_{35}H_{36}N_2O_9$, M_{calc} = 628.2421).

3.3.8. Thapoxepine A (+)-(2R,3 S,4R,2′RS)-1-[2,3,4,5-Tetrahydro-2-methoxycarbonyl-2-(4-methoxyphenyl)-6-methoxy-7,8-methylenedioxy-5-oxo-3-phenyl-1-benzoxepin-4-carbonyl]-2-(2-methylpropanoylamino)-pyrrolidine (**9**)

Epimeric mixture of (2′*S*)-**9A**: (2′*R*)-**9B** = 2:1. $[\alpha]_D^{20}$ = +55° (*c* = 0.6, $CHCl_3$). UV λ^{MeOH} nm (log ϵ): 231 (*sh*, 4.38), 249 (*sh*, 4.19), 280 (3.90), 297 (*sh*, 3.78), 335 (3.38). IR ν^{CCl_4} cm^{-1}: 3494 *w*, 3426 *w*, 3336 *w*, 2968 *m*, 2934 *m*, 2878 *w*, 1760 *m*, 1682 *s*, 1658 *m*, 1624 *m*, 1512 *s*, 1500 *m*, 1470 *s*, 1420 *m*, 1256 *m*, 1223 *s*, 1216 *s*, 1180 *m*, 1160 *w*, 1092 *m*, 1072 *m*, 1038 *w*, 1018 *w*, 966 *w*, 945 *w*, 934 *w*, 700 *m*. ^1H and ^{13}C NMR see Tables 1 and 2. HMBC: 3-H (2 *w*, 4, 11, 1″, 2′6′ or 2″6″), 4-H (3, 5, 11, 1″), 9-H (5a, 7, 8, 9a), 19-H (18, 20, 21), 20 + 21-H_3 (18, 19, 20, 21), 2′6′-H (2, 4′), 3′5′-H (1′, 4′), 2″6″-H (3″5″, 4″), 3″-5″-H (1″), 6-OCH_3 (6), 4′-OCH_3 (4′), OCH_2O (7), 10-$OC\underline{H}_3$ (10). NOESY: (**3A**): 3-H (2′6′, 2″6″), 4-H (13, 2″6″), 13-H (4, 14, 15), 14 + 15-H (13, 16, NH), 16-H (14, 15), 19-H (20, 21, NH), 20 + 21-H_3 (19), 2′6′-H (3, 3′5′), 3′5′-H (2′6′, 4′-OCH_3), 2″6″-H (3, 4, 13 *w*), 10-OCH_3 (2″6″), 4′-OCH_3 (3′5′), NH (14, 15, 19, 20). EI-MS (70 eV, 200°C) *m/z*: 658 (3%) M^+, 571 (5), 502 (60), 443 (100), 308 (17), 280 (11), 265 (11), 221 (26), 195 (66), 180 (14), 165 (12), 135 (27), 121 (23), 85 (15), 70 (59); FD-MS *m/z*: 658 ($C_{36}H_{38}N_2O_{10}$, M_{calc} = 658.2526).

3.3.9. Homothapoxepine A (+)-(2R,3 S,4R,2′S)-1-[2,3,4,5-Tetrahydro-2-methoxycarbonyl-2-(4-methoxyphenyl)-6-methoxy-7,8-methylenedioxy-5-oxo-3-phenyl-1-benzoxepin-4-carbonyl]-2-(2-methylbutanoylamino)-pyrrolidine (**10**)

$[\alpha]_D^{20}$ = +110° (*c* = 0.4, $CHCl_3$). UV λ^{MeOH} nm (log ϵ): 231 (*sh*, 4.42), 249 (*sh*, 4.19), 280 (3.90), 297 (*sh*, 3.78), 334 (3.48). IR ν^{CHCl_3} cm^{-1}: 3500 *w*, 3420 *w*, 2952 *m*, 2932 *m*, 2874 *w*, 1754 *m*, 1670 *m*, 1644 *m*, 1626 *s*, 1610 *m*, 1512 *m*, 1500 *w*, 1470 *s*, 1420 *m*, 1381 *w*, 1307 *w*, 1256 *m*, 1180 *m*, 1162 *w*, 1094 *m*, 1016 *w*, 1088 *w*, 966 *w*. ^1H and ^{13}C NMR see Tables 1 and 2. HMBC: 3-H (4, 1″), 4-H (5), 9-H (7, 8), 21-H_3 (19, 20), 22-H_3 (18), 2′6′-H (2, 4′), 3′5′ (4′), 6-OCH_3 (6), 4′-OCH_3 (4′), 10-$OC\underline{H}_3$ (10). NOESY: 3-H (2′6′, 2″6″), 4-H (2″6″), 13-H (4, 15), 16-H (15), 19-H (22, NH), 2′6′-H (3), 3′5′-H (4′-OCH_3), 2″6″-H (3, 4), 4′-OCH_3 (3′5′), NH (19). EI-MS (70 eV, 200°C) *m/z*: 672 (8%) M^+, 502 (45), 443 (54), 285 (11), 256 (20), 221 (11), 205 (17), 195 (21), 163 (12), 135 (52), 129 (23), 121 (21), 109 (27), 95 (25), 85 (43), 69 (69), 57 (100); FD-MS *m/z*: 672 ($C_{32}H_{40}N_2O_{10}$, M_{calc} = 672.2683); M_{exp} = 672.2683.

3.3.10. Thapoxepine B (**11**) (+)-(2R,3R,4 S,2′RS)-1-[2,3,4,5-Tetrahydro-2-methoxycarbonyl-2-(4-methoxyphenyl)-6-methoxy-7,8-methylenedioxy-5-oxo-3-phenyl-1-benzoxepin-4-carbonyl]-2-(2-methylpropanoylamino)-pyrrolidine (**11**)

Epimeric mixture of (2′*S*)-**11A**: (2′*R*)-**11B** = 5:4. mp. 260 to 262°. $[\alpha]_D^{20}$ = +33° (*c* = 0.5, $CHCl_3$). UV λ^{MeOH} nm (log ϵ): 231 (*sh*, 4.23), 249 (*sh*, 4.04), 281 (3.81), 297 (*sh*, 3.73), 336 (3.43). IR ν^{CHCl_3} cm^{-1}: 3512 *w*, 3430 *w*, 2950 *w*, 2838 *w*, 1754 *m*, 1676 *s*, 1654 *s*, 1622 *m*, 1606 *m*, 1520 *m*, 1500 *m*, 1470 *s*, 1420 *m*, 1372 *w*, 1256 *m*, 1182 *w*, 1162 *w*, 1104 *m*, 1016 *w*, 966 *w*, 945 *w*. ^1H and ^{13}C NMR see Tables 1 and 2. NOESY: (**11a**): 3-H (2′6′, 2″6″), 4-H (13, 2″6″), 19 (13, 20, 21), 20 + 21-H_3 (19, for **11B** additionally 2″6″), 4-OCH_3 (3′5′), 2′6′-H (9, 3′5′), 3′5′-H (2′6′, 4′-OCH_3), 2″6″-H (3, 4, 10-OCH_3), 10-OCH_3 (2″6″). EI-MS (70 eV, 200°C) *m/z*: 658 (3%) M^+, 571 (4), 502 (65), 443 (100), 308 (19), 280 (10), 265 (11), 221 (36), 195 (61),

180 (15), 165 (10), 135 (32), 121 (23), 85 (20), 70 (73); FD-MS m/z: 658 ($C_{36}H_{38}N_2O_{10}$, M_{calc} = 658.2526).

3.4. Hydrolysis of thapsakin A acetate

Thapsakin A acetate (**6**) hydrolyzed easily in protic solvents (MeOH) during prep TLC workup to give the corresponding 10-hydroxy compound **12** (thapsakin A). ^{1}H NMR ($CDCl_3$) δ = 4.48 (*d*, 1H, *J* = 9.4 Hz, 3-H), 4.16 (*d*, 1H, *J* = 9.4 Hz, 4-H), 6.15 (*s*, 1H, 9-H), 4.77 (*s*, 1H, 10-H), 6.45 (*br.dd*, 1H, *J* = 7.5) and ca 6 Hz, 13-H), 1.70 and 1.90 (2 × *m*, 4H, 14 + 15-H_2), 3.35 and 3.55 (2 × *m*, 2H, 16-H a and b), 2.02 (*sept*, 1H, *J* = 6.9 Hz, 19-H), 0.90 and 0.98 (2 × *d*, 2 × 3H, *J* = 6.9 Hz, 20- and 21-H_3), 7.39 (*d*, 2H, *J* = 8.9 Hz, 2′ + 6′-H), 6.63 (*d*, 2H, *J* = 8.9 Hz, 3′ + 5′-H), 7.13 (*m*, 2H, 2″ + 6″-H), 6.90 to 7.05 (*m*, 3H, 3″ + 5″- and 4″-H), 4.01 (*s*, 3H, 6-OCH_3), 3.68 (*s*, 3H, 4′-OCH_3), 5.86 (*s*, 2H, OCH_2O), 5.83 (*s*, 1H, 5-OH), 4.12 (*s*, 1H, 10-OH), 5.17 (*s*, 1H, NH). ^{13}C NMR ($CDCl_3$) δ = 88.6 (*s*, C-2), 57.3 (*d*, C-3), 62.9 (*d*, C-4), 82.2 (*s*, C-5), 107.3 (*s*, C-5a), 140.2 (*s*, C-6), 129.7 (*s*, C-7), 149.6 (*s*, C-8), 93.6 (*d*, C-9), 147.5 (*s*, 9a), 79.8 (*d*, C-10), 169.7 (*s*, C-11), 63.4 (*d*, C-13), 34.4 (*t*, C-14), 21.2 (*t*, C-15), 46.00 (*t*, C-16), 175.7 (*s*, C-18), 35.00 (*d*, C-19), 19.5 and 18.8 (2 × *q*, 20- and 21-C), 130.3 and 130.1 (2 × *d*, 2′6′ and 2″6″), 112.8 (*d*, 3′5′), 158.6 (*s*, 4′), 141.9 (*s*, 1″), 127.8 (*d*, 3″5″), 125.8 (*d*, 4″), 60.2 (*q*, 6-OCH_3), 55.0 (*q*, 4′-OCH_3), 101.0 (*t*, OCH_2O).

3.5. Insect bioassays

Larvae of *S. littoralis* were from a laboratory colony reared on a bean based artificial diet (Srivastava & Proksch, 1991). The chronic feeding bioassays were conducted with freshly hatched larvae (n = 20) that were kept on artificial diet spiked with different concentrations of the test compounds (0.05 to 50 μg/g fr. wt.), which were applicated with Me_2CO. After 5 days (moist chamber, 29°C, darkness) the survival rate and the larval growth of the surviving larvae were monitored in comparison to the control treated with solvent only. Rocaglamide, aglafolin (= methyl rocaglate) and rocaglaol (isolated from the roots of *A. odorata* Lour. collected in Ko Samet, southeast Thailand, HG 501), as well as commercial azadirachtin (> 96%) from Roth (Karlsruhe, Germany), were used for comparison. From the dose response curves in each experiment (three replicates) LC_{50} and EC_{50} values were calculated by probit-log analysis (Table 3).

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