Dioncophylline A as a Growth-Retarding Agent against the Herbivorous Insect *Spodoptera littoralis*: Structure−Activity Relationships[†]

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Dioncophylline A (**1**) represents a novel insecticidal agent, as documented by its enhanced growth-retarding effect on larvae of the polyphagous pest insect *Spodoptera littoralis*. Within the scope of the work described here, the potential of this as yet most active naphthylisoquinoline alkaloid was further elucidated by the preparation and testing of selected analogues. Investigation of a broad series of structurally modified dioncophylline A analogues (**2**−**20**) revealed the free amine function to be essential for the growth inhibitory effect, whereas a modification of the OH function partially led to a distinct increase of activity. In particular, the 8-*O*-alkyl (especially 8-*O*-benzylated) derivatives (**14** and **15** as well as **16**−**19**) displayed pronounced effects. In the case of 8-*O*-(*p*-bromobenzyl)dioncophylline A (**16**), the activity of the natural parent compound dioncophylline A (**1**) (EC$_{50}$ = 277 μg/g fresh wt of diet; concentration that inhibits larval growth by 50%) was even improved by a factor of >15 (EC$_{50}$ = 15.6 μg/g fresh wt).

Monomeric and dimeric naphthylisoquinoline alkaloids[1] represent the main secondary metabolites of tropical lianas of the families Ancistrocladaceae and Dioncophyllaceae, which are endemic to the tropical rainforests of West as well as Middle and East Africa, India, and Southeast Asia.[2] These intriguing natural products are characterized by their unprecedented acetogenic origin and their broad structural variability, including the presence of a sterically hindered (and thus stereogenic) biaryl axis and two stereocenters. They display a wide range of interesting pharmacological properties.[1] Thus, michellamines, dimeric naphthylisoquinoline alkaloids isolated from the Cameroonian liana *Ancistrocladus korupensis* (Ancistrocladaceae), have been shown to exhibit outstanding antiviral properties against HIV-I and -II,[3,4] whereas several "monomeric" naphthylisoquinolines revealed excellent antimalarial activities against *Plasmodium falciparum* and *Plasmodium berghei* in vitro.[5,6] Dioncophylline A (**1**), one of the main alkaloids of *Triphyophyllum peltatum*[7] (Dioncophyllaceae) and several *Ancistrocladus* species,[1,8,9] was shown to display, besides fungicidal,[10] molluscicidal,[11] and larvicidal[12] properties, pronounced feeding deterrency and growth-retarding activity against larvae of the polyphagous herbivore *Spodoptera littoralis*, thus representing a novel insecticidal lead.[13−15] Here we describe the first structure−activity investigations, leading to the detection of structural analogues of **1** that show distinctly enhanced activities compared to the parent compound.

Results and Discussion

Dioncophylline A (**1**) displays a wide range of principal structural variation possibilities, including the protection of functional groups, configurative changes at stereogenic centers or at the axis, and further functionalizations of aromatic or side-chain positions. In a first approach to structure−activity investigations on this as yet most active naphthylisoquinoline alkaloid, we focused on the importance of the free amine and the 8-OH function for the growth-retarding activity toward *S. littoralis*.

Thus, a broad series of *N*- and 8-*O*-substituted dioncophylline A analogues was prepared and tested for their growth-retarding and feeding-deterrency activities against larvae of *S. littoralis*. The growth-inhibitory effects expressed as EC$_{50}$ (concentration that inhibits larval growth by 50%) are presented in Table 1. No influence of dioncophylline A (**1**) or any of its derivatives on the survival rate of the larvae was detected within the performed tests.

As a first result, *N*-derivatization (**2**−**4**) both by acylation or alkylation led to a significant decrease of activity as compared to the natural parent compound **1**, whereas a derivatization of the 8-OH function significantly enhanced the growth-retarding potential, in some cases, interestingly, even for the *N*-formylated derivatives **5**−**8**. The 8-*O*-acylated analogues **9**−**12** displayed lower activities than dioncophylline A (**1**). By contrast—compared to **2**—derivatives **10**, **11**, and especially **13** showed a significant increase of activity, the last being even more active than the natural precedent **1**. Based on these results, two selected, exclusively 8-*O*-alkylated derivatives, **14** and **15**, of dioncophylline A (**1**) were synthesized. Both derivatives displayed higher activities (see Table 1) than the corresponding *N*-formyl analogues **5** and **6**, underlining the above-stated finding that the free secondary amine function favorably influences the activity. The most active derivative, 8-*O*-
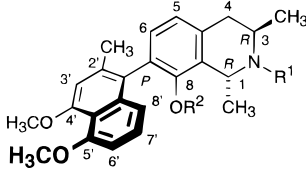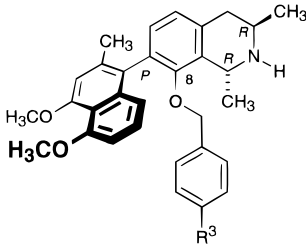
Table 1. Growth-Retarding Activities of Dioncophylline A (**1**) and Its Derivatives, **2−15**, against Larvae of *S. littoralis*

	R[1]	R[2]	EC$_{50}$ (μg/g fresh wt)
1	H	H	277
2	CHO	H	454
3	COCH$_3$	H	291
4	CH$_3$	H	377
5	CHO	CH(CH$_3$)$_2$	130
6	CHO	CH$_2$(C$_6$H$_5$)	67.1
7	CHO	(CH$_2$)$_3$CH$_3$	182
8	CHO	CH$_3$	171
9	COCH$_3$	COCH$_3$	331
10	CHO	COCH$_3$	361
11	CHO	COC(CH$_3$)$_3$	333
12	CHO	CO(CH$_2$)$_{11}$CH$_3$	>750
13	CHO	CO(C$_6$H$_5$)	132
14	H	CH(CH$_3$)$_2$	94.1
15	H	CH$_2$(C$_6$H$_5$)	<30.0

Table 2. Growth-Inhibitory Effects of 8-*O*-Benzylated Dioncophylline A Derivatives **15−19**

	R[3]	EC$_{50}$ (μg/g fresh wt)
15	H	27.1
16	Br	15.6
17	Cl	16.4
18	NO$_2$	25.6
19	CF$_3$	35.1

benzyldioncophylline A (**15**), was found to exceed even the "parent" compound dioncophylline A (**1**) by a factor of ca. 10.

Because of the high activity of **15**, a further, more detailed modification by the investigation of differently substituted 8-*O*-benzyl groups seemed worth investigation. Three of the derivatives thus prepared, **16−18**, yielded even higher activities than **15** (see Table 2). Among them, 8-*O*-(*p*-bromobenzyl)dioncophylline A (**16**) was the most active (EC$_{50}$ = 15.6 μg/g fresh wt) dioncophylline A derivative, its activity surpassing that of **1** (EC$_{50}$ = 277 μg/g fresh wt) by a factor of more than 15. Likewise excellent is the activity of the corresponding *p*-chlorobenzyl derivative **17**, which has virtually the same EC$_{50}$ value (16.4 μg/g fresh wt) as **16**. Regrettably, the chosen synthetic strategy did not allow for the synthesis of analogous compounds with electron-richer (e.g., *p*-methoxy substituted) benzyl groups, because the *N*-formyl groups could not be removed without cleavage of the 8-*O*-benzyl groups.

Given the positive influence of a bromo substituent in the 8-*O*-benzyl group, we introduced bromine into the aromatic 5-position of the tetrahydroisoquinoline moiety of dioncophylline A (**1**), to give compound **20** (EC$_{50}$ =

193 μg/g fresh wt), which, however, did not greatly influence the activity compared with **1** (EC$_{50}$ = 277 μg/g fresh wt).
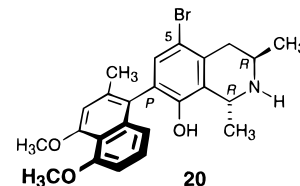
The free amine function of dioncophylline A (**1**) represents an important precondition for a strong inhibition of the growth of *S. littoralis*, whereas derivatization of the 8-OH function with an isopropyl and especially with a benzyl group leads to a pronounced enhancement of the growth inhibition (cf. **15**,**16**). The incorporation of (electron-withdrawing) substituents into the benzyl moiety of 8-*O*-benzyldioncophylline A (**15**) causes the strongest effects, with 8-*O*-(*p*-bromobenzyl)-dioncophylline A (**16**) being more than 15 times more active than **1** itself, whereas a bromination of the tetrahydroisoquinoline in the 5-position (cf. **20**) does not significantly influence the activity.

The excellent activities of the derivatives **16−19** suggest future research activities to explore the scope of dioncophylline A (**1**) as an insecticidal agent; for example, by the preparation of further analogues related to **16−19**, but also by the investigation of the mode of action and the metabolism of this active compound.

Experimental Section

General Experimental Procedures. Melting points were determined on a Reichert-Jung Thermovar hotplate and are uncorrected. Optical rotations were taken on a Perkin-Elmer 241 polarimeter; IR spectra, on a Perkin-Elmer 1429 spectrophotometer. ^{1}H-NMR spectra were measured on Bruker AC 200 (200 MHz), Bruker AC 250 (250 MHz), and Bruker DMX 600 (600 MHz) instruments using CDCl$_3$ as solvent and internal standard (δ 7.26). EIMS were measured on Finnigan MAT 8200 and Finnigan MAT 90 instruments. Reactions were monitored by TLC using plates of Si gel (0.063−0.200 mm, Merck), which were deactivated with gaseous NH$_3$. Column chromatography was carried out using Si gel (0.063−0.200, Merck), deactivated with 5% concd NH$_3$. All solvents and acylhalides were distilled prior use. Dioncophylline A (**1**) was available from previous isolation work.[7] *N*-Methyldioncophylline A (**4**)[16] and *N*-formyl-8-*O*-methyldioncophylline A (**8**)[17] were prepared as described elsewhere.

Synthesis of New Compounds. Transformation of Amines into the Corresponding Hydrochlorides. To a solution of the substrate in MeOH was carefully added cold saturated methanolic HCl at 0 °C in excess. After removal of the solvent *in vacuo*, the hydrochloride was recrystallized from the given solvent.

***N*-Formyldioncophylline A (2).**[18] A mixture of 2.00 g (5.30 mmol) dioncophylline A (**1**) and 948 μL (759 mg, 5.83 mmol) of pivalic formic anhydride[19] in 100 mL of CH$_2$Cl$_2$ was stirred at 20 °C for 1 h. Removal of the solvent *in vacuo* afforded a pale yellow solid, which was recrystallized from CHCl$_3$−petroleum ether to give **2** (2.11 g, 98%) as colorless crystals: mp 233 °C; [α]^{20}$_D$

+21.3° (c 0.42, CHCl$_3$); IR (KBr) ν_{max} 3410 (br, OH), 2955 (m, CH), 2916 (m, CH), 1636 (s, C=O), 1600 (m, C=C), 1579 (s, C=C), 1381 (s, CH$_3$), 1252 (s, C–O), 752 (m, CH) cm^{-1}; ^{1}H NMR (600 MHz) 1:1.6 mixture of interconverting rotational isomers with respect to the *N*-formyl bond, major isomer δ 1.40 (3H, d, J = 6.7 Hz, 3-CH$_3$), 1.48 (3H, d, J = 6.7 Hz, 1-CH$_3$), 2.19 (3H, s, 2′-CH$_3$), 2.76 (1H, dd, J = 15.5, 7.0 Hz, 4-H$_{ax}$), 3.16 (1H, dd, J = 15.5, 4.1 Hz, 4-H$_{eq}$), 3.96 (3H, s, 4′- or 5′-OCH$_3$), 4.00 (3H, s, 5′- or 4′-OCH$_3$), 4.14 (1H, m$_c$, 3-H), 5.72 (1H, q, J = 6.5 Hz, 1-H), 6.79–6.98 (5H, m, arom H), 7.24 (1H, t, J = 8.0 Hz, 7′-H), 8.36 (1H, s, CHO); minor isomer δ 1.12 (3H, d, J = 6.2 Hz, 3-CH$_3$), 1.50 (3H, d, J = 6.7 Hz, 1-CH$_3$), 2.20 (3H, s, 2′-CH$_3$), 2.71 (1H, dd, J = 15.0, 0.7 Hz, 4-H$_{ax}$), 3.25 (1H, dd, J = 15.0, 5.4 Hz, 4-H$_{eq}$), 3.97 (3H, s, 4′- or 5′-OCH$_3$), 4.01 (3H, s, 5′- or 4′-OCH$_3$), 4.70 (1H, m$_c$, 3-H), 5.21 (1H, q, J = 6.6 Hz, 1-H), 6.79–6.98 (5H, m, arom H), 7.26 (1H, t, J = 8.1 Hz, 7′-H), 8.38 (1H, s, CHO); EIMS (70 eV) m/z 405 (51) [M$^+$], 390 (91) [M$^+$ − CH$_3$], 376 (100) [M$^+$ − CHO], 362 (31) [M$^+$ − C$_2$H$_3$O]; HREIMS m/z 405.1939 (calcd for C$_{25}$H$_{27}$NO$_4$, 405.1940).

N-Acetyldioncophylline A (3). A mixture of 200 mg (0.530 mmol) dioncophylline A (**1**), 74 μL (53.6 mg, 0.530 mmol) triethylamine, a catalytic amount of 4-dimethylaminopyridine (DMAP), and 38 μL (41.6 mg, 0.530 mmol) acetyl chloride in 30 mL of CH$_2$Cl$_2$ was stirred at 20 °C for 1 h. After treatment with 10 mL of 2 M aqueous NH$_4$Cl, the organic layer was chromatographed (CH$_2$Cl$_2$–MeOH 9:1) on Si gel. Recrystallization from CH$_2$Cl$_2$–Et$_2$O afforded **3** (222 mg, 76%) as colorless crystals: mp 237 °C; [α]$^{20}_D$ +53.8° (c 0.50, CHCl$_3$); IR (KBr) ν_{max} 3412 (m, OH), 2950 (m, CH), 2913 (m, CH), 1622 (s, C=O), 1582 (m, C=C), 1386 (s, CH$_3$), 1255 (s, C–O), 751 (m, CH) cm^{-1}; ^{1}H NMR (600 MHz) 1:1.1 mixture of interconverting rotational isomers with respect to the *N*-acetyl bond, major isomer δ 1.06 (3H, d, J = 6.2 Hz, 3-CH$_3$), 1.46 (3H, d, J = 6.6 Hz, 1-CH$_3$), 2.18 (3H, s, 2′-CH$_3$), 2.27 (3H, s, COCH$_3$), 2.67 (1H, dd, J = 14.9, 1.2 Hz, 4-H$_{ax}$), 3.27 (1H, dd, J = 14.7, 5.3 Hz, 4-H$_{eq}$), 3.95 (3H, s, 4′- or 5′-OCH$_3$), 4.00 (3H, s, 5′- or 4′-OCH$_3$), 4.82 (1H, m$_c$, 3-H), 5.30 (1H, q, J = 6.5 Hz, 1-H), 6.78–6.95 (5H, m, arom H), 7.23 (1H, t, J = 8.1 Hz, 7′-H); minor isomer δ 1.11 (3H, d, J = 6.3 Hz, 3-CH$_3$), 1.41 (3H, d, J = 6.4 Hz, 1-CH$_3$), 2.18 (3H, s, 2′-CH$_3$), 2.24 (3H, s, COCH$_3$), 2.69 (1H, dd, J = 13.9, 1.6 Hz, 4-H$_{ax}$), 3.36 (1H, dd, J = 14.8, 4.3 Hz, 4-H$_{eq}$), 3.94 (3H, s, 4′- or 5′-OCH$_3$), 3.99 (3H, s, 5′- or 4′-OCH$_3$), 4.38 (1H, m$_c$, 3-H), 5.65 (1H, q, J = 6.5 Hz, 1-H), 6.78–6.95 (5H, m, arom H), 7.25 (1H, t, J = 7.8 Hz, 7′-H); EIMS (70 eV) m/z 419 (60) [M$^+$], 404 (83) [M$^+$ − CH$_3$], 362 (100) [M$^+$ − C$_3$H$_5$O]; HREIMS m/z 419.2093 (calcd for C$_{26}$H$_{29}$NO$_4$, 419.2097).

General Procedure for the Synthesis of 8-O-Alkyl-N-formyldioncophylline A Derivatives 5–7. To a suspension of **2** and 1.5 equiv of K$_2$CO$_3$ in Me$_2$CO (20–40 mL) was added an excessive amount (3 equiv) of the corresponding alkyl halide with stirring, and the reaction mixture was heated to reflux. After completion of the reaction (48–96 h, monitored by TLC), the solvent was removed *in vacuo*, and the residue was chromatographed on Si gel (CH$_2$Cl$_2$).

N-Formyl-8-O-isopropyldioncophylline A (5). Compound **2** (200 mg, 0.493 mmol) was treated as described above with 102 mg (0.740 mmol) of K$_2$CO$_3$ and

139 μL (182 mg, 1.48 mmol) of isopropyl bromide to give **5** (196 mg, 89%) as pale brown, thick oil: [α]$^{20}_D$ +12.7° (c 0.50, CHCl$_3$); IR (KBr) ν_{max} 2960 (m, CH), 2918 (m, CH), 1653 (s, C=O), 1585 (s, C=C), 1375 (s, CH$_3$), 756 (m, CH) cm^{-1}; ^{1}H NMR (200 MHz) main rotational isomer with respect to the *N*-formyl bond δ 0.73 [3H, d, J = 6.1 Hz, CH(CH$_3$)CH_3], 0.82 [3H, d, J = 6.1 Hz, CH-(CH_3)CH$_3$], 1.33 (3H, d, J = 6.6 Hz, 3-CH$_3$), 1.42 (3H, d, J = 6.6 Hz, 1-CH$_3$), 2.17 (3H, s, 2′-CH$_3$), 2.75 (1H, dd, J = 15.6, 5.8 Hz, 4-H$_{ax}$), 3.19 (1H, dd, J = 15.6, 4.1 Hz, 4-H$_{eq}$), 3.43 [1H, m, CH(CH$_3$)CH$_3$], 3.99 (3H, s, 4′- or 5′-OCH$_3$), 4.01 (3H, s, 5′- or 4′-OCH$_3$), 4.16 (1H, m$_c$, 3-H), 5.73 (1H, q, J = 6.9 Hz, 1-H), 6.72–7.30 (6H, m, arom H), 8.36 (1H, s, CHO); EIMS (70 eV) m/z 447 (40) [M$^+$], 432 (5) [M$^+$ − CH$_3$], 405 (16) [M$^+$ − C$_3$H$_6$], 390 (69) [M$^+$ − C$_4$H$_9$], 376 (10) [M$^+$ − C$_4$H$_7$O], 59 (58) [C$_3$H$_7$O$^+$], 43 (100) [C$_3$H$_7^+$]; HREIMS m/z 447.2415 (calcd for C$_{28}$H$_{33}$NO$_4$, 447.2410).

8-O-Benzyl-N-formyldioncophylline A (6). In analogy to the general reaction procedure, 200 mg (0.493 mmol) of **2** was treated with 102 mg (0.740 mmol) of K$_2$CO$_3$ and 175 μL (253 mg, 1.48 mmol) of benzyl bromide to give **6**, recrystallized from Et$_2$O, as colorless crystals (210 mg, 86%): mp 58 °C; [α]$^{20}_D$ −45.0° (c 0.50, CHCl$_3$); IR (KBr) ν_{max} 2950 (m, CH), 2910 (m, CH), 1650 (s, C=O), 1581 (m, C=C), 1381 (s, CH$_3$), 1253 (m, C–O), 749 (w, CH) cm^{-1}; ^{1}H NMR (200 MHz) main rotational isomer with respect to the *N*-formyl bond δ 1.36 (3H, d, J = 6.6 Hz, 3-CH$_3$), 1.45 (3H, d, J = 6.6 Hz, 1-CH$_3$), 2.18 (3H, s, 2′-CH$_3$), 2.77 (1H, dd, J = 15.8, 6.6 Hz, 4-H$_{ax}$), 3.20 (1H, dd, J = 15.6, 5.8 Hz, 4-H$_{eq}$), 3.99 (3H, s, 4′- or 5′-OCH$_3$), 4.01 (3H, s, 5′- or 4′-OCH$_3$), 4.16 (1H, m$_c$, 3-H), 4.17 [1H, d, J = 10.6 Hz, C(H)H(C$_6$H$_5$)], 4.38 [1H, d, J = 10.9 Hz, C(H)H(C$_6$H$_5$)], 5.68 (1H, q, J = 6.6 Hz, 1-H), 6.78–7.31 (11H, m, arom H), 8.34 (1H, s, CHO); EIMS (70 eV) m/z 495 (100) [M$^+$], 480 (44) [M$^+$ − CH$_3$], 466 (5) [M$^+$ − CHO], 404 (48) [M$^+$ − C$_7$H$_7$], 389 (41) [M$^+$ − C$_8$H$_{10}$]; HREIMS m/z 495.2408 (calcd for C$_{32}$H$_{33}$NO$_4$, 495.2409).

8-O-n-Butyl-N-formyldioncophylline A (7). According to the above-described reaction conditions, 100 mg (0.247 mmol) of **2** was converted to **7** (100 mg, 88%) with 51.3 mg (0.371 mmol) of K$_2$CO$_3$ and 77 μL (68.6 mg, 0.741 mmol) of 1-chlorobutane. Compound **7** was obtained as a pale yellow oil: [α]$^{20}_D$ +26.9° (c 0.50, CHCl$_3$); IR (CCl$_4$) ν_{max} 2957 (m, CH), 2917 (m, CH), 1662 (s, C=O), 1575 (m, C=C), 1381 (s, CH$_3$), 1253 (m, C–O) cm^{-1}; ^{1}H NMR (200 MHz) main rotational isomer with respect to the *N*-formyl bond δ 0.53 (3H, t, J = 6.9 Hz, CH$_2$CH$_2$CH$_2$CH_3), 0.77–1.04 (2H, m, CH$_2$CH$_2$CH_2CH$_3$), 1.05–1.25 (2H, m, CH$_2$CH_2CH$_2$CH$_3$), 1.38 (3H, d, J = 6.6 Hz, 3-CH$_3$), 1.47 (3H, d, J = 6.6 Hz, 1-CH$_3$), 2.18 (3H, s, 2′-CH$_3$), 2.76 (1H, dd, J = 15.9, 6.9 Hz, 4-H$_{ax}$), 3.17 (1H, dd, J = 17.6, 6.0 Hz, 4-H$_{eq}$), 3.18–3.44 (2H, m, CH_2CH$_2$CH$_2$CH$_3$), 3.99 (3H, s, 4′- or 5′-OCH$_3$), 4.01 (3H, s, 5′- or 4′-OCH$_3$), 4.14 (1H, m$_c$, 3-H), 5.67 (1H, q, J = 6.8 Hz, 1-H), 6.79–7.24 (6H, m, arom H), 8.37 (1H, s, CHO); EIMS (70 eV) m/z 461 (100) [M$^+$], 446 (42) [M$^+$ − CH$_3$], 432 (8) [M$^+$ − CHO], 404 (4) [M$^+$ − C$_4$H$_9$], 390 (43) [M$^+$ − C$_5$H$_{11}$]; HREIMS m/z 461.2565 (calcd for C$_{29}$H$_{35}$NO$_4$, 461.2566).

General Procedure for the Synthesis of N-Acyl-8-O-acyldioncophylline A Derivatives 9–13. A mixture of the corresponding N-acyldioncophylline A (**2** or **3**), 1.2 equiv triethylamine, a catalytic amount of

4-(dimethylamino)pyridine (DMAP), and 1.2 equiv of the corresponding acyl chloride in 20 mL of CH_2Cl_2 was stirred at 20 °C for 1 h. After addition of 5 mL of a 1 M aqueous NH_4Cl, the organic layer was chromatographed on Si gel (CH_2Cl_2). Recrystallization from CH_2Cl_2- petroleum ether afforded derivatives **9–13**.

N-Acetyl-8-O-acetyldioncophylline A (9). Compound **3** (100 mg, 0.238 mmol) was treated as described above with 39 μL (28.9 mg, 0.286 mmol) of triethylamine, 20 μL (22.4 mg, 0.286 mmol) of acetyl chloride, and a catalytic amount of DMAP to give **9** (84.6 mg, 77%) as colorless crystals: mp 101 °C; $[\alpha]^{20}_D$ +139.2° (c 0.50, CHCl₃); IR (KBr) ν_{max} 2955 (m, CH), 2917 (m, CH), 1751 (m, C=O, O-acetyl), 1623 (s, C=O, N-acetyl), 1582 (s, C=C), 1370 (s, CH₃), 1256 (m, C−O), 1187 (s, C−O), 750 (w, CH) cm⁻¹; ¹H NMR (200 MHz) main rotational isomer with respect to the N-acetyl bond δ 1.09 (3H, d, J = 6.7 Hz, 3-CH₃), 1.36 (3H, d, J = 6.6 Hz, 1-CH₃), 1.54 (3H, s, OCOCH₃), 2.19 (3H, s, 2′-CH₃), 2.25 (3H, s, NCOCH₃), 2.77 (1H, dd, J = 15.2, 2.4 Hz, 4-H$_{ax}$), 3.42 (1H, dd, J = 14.7, 4.5 Hz, 4-H$_{eq}$), 3.98 (3H, s, 4′- or 5′-OCH₃), 4.00 (3H, s, 5′- or 4′-OCH₃), 4.39 (1H, m$_c$, 3-H), 5.41 (1H, q, J = 6.0 Hz, 1-H), 6.75−7.25 (6H, m, arom H); EIMS (70 eV) m/z 461 (92) [M⁺], 446 (7) [M⁺ − CH₃], 419 (9) [M⁺ − C₂H₂O], 404 (100) [M⁺ − C₃H₅O], 376 (9) [M⁺ − C₄H₅O₂], 362 (74) [M⁺ − C₅H₇O₂]; HREIMS m/z 461.2202 (calcd for C₂₈H₃₁NO₅, 461.2202).

8-O-Acetyl-N-formyldioncophylline A (10). According to the general procedure, 100 mg (0.247 mmol) of **2** was converted to **10** (88.4 mg, 80%) with 41 μL (30.0 mg, 0.296 mmol) of triethylamine, 21 μL (23.3 mg, 0.296 mmol) of acetyl chloride, and a catalytic amount of DMAP. Compound **10** was obtained as colorless crystals: mp 184 °C; $[\alpha]^{20}_D$ −44.5° (c 0.50, CHCl₃); IR (KBr) ν_{max} 2950 (m, CH), 2917 (m, CH), 1751 (m, C=O), 1648 (s, C=O, acetyl), 1635 (s, C=O, formyl), 1582 (s, C=C), 1386 (s, CH₃), 1256 (s, C−O), 1186 (s, C−O), 745 (w, CH) cm⁻¹; ¹H NMR (200 MHz) main rotational isomer with respect to the N-formyl bond δ 1.33 (3H, d, J = 6.7 Hz, 3-CH₃), 1.41 (3H, d, J = 6.6 Hz, 1-CH₃), 1.54 (3H, s, COCH₃), 2.19 (3H, s, 2′-CH₃), 2.81 (1H, dd, J = 15.5, 5.7 Hz, 4-H$_{ax}$), 3.26 (1H, dd, J = 15.9, 4.8 Hz, 4-H$_{eq}$), 3.98 (3H, s, 4′- or 5′-OCH₃), 4.00 (3H, s, 5′- or 4′-OCH₃), 4.19 (1H, m$_c$, 3-H), 5.47 (1H, q, J = 6.8 Hz, 1-H), 6.76−7.22 (6H, m, arom H), 8.35 (1H, s, CHO); EIMS (70 eV) m/z 447 (75) [M⁺], 405 (29) [M⁺ − C₂H₂O], 390 (100) [M⁺ − C₃H₅O]; HREIMS m/z 447.2037 (calcd for C₂₇H₂₉NO₅, 447.2046).

N-Formyl-8-O-pivaloyldioncophylline A (11). According to the general procedure, 100 mg (0.247 mmol) of **2** was treated with 41 μL (30.0 mg, 0.296 mmol) of triethylamine, 37 μL (35.5 mg, 0.296 mmol) of pivaloyl chloride, and a catalytic amount of DMAP to give **11** (89.5 mg, 74%) as colorless needles: mp 208 °C; $[\alpha]^{20}_D$ −44.0° (c 0.50, CHCl₃); IR (KBr) ν_{max} 2950 (m, CH), 2901 (m, CH), 1732 (m, C=O, pivaloyl), 1652 (s, C=O, formyl), 1568 (m, C=C), 1375 (s, CH₃), 1261 (s, C−O), 1113 (s, C−O), 752 (m, CH) cm⁻¹; ¹H NMR (200 MHz) main rotational isomer with respect to the N-formyl bond δ 0.67 (9H, s, CO(CH₃)₃), 1.33 (3H, d, J = 6.6 Hz, 3-CH₃), 1.43 (3H, d, J = 6.7 Hz, 1-CH₃), 2.19 (3H, s, 2′-CH₃), 2.81 (1H, dd, J = 15.3, 5.7 Hz, 4-H$_{ax}$), 3.31 (1H, dd, J = 14.1, 5.0 Hz, 4-H$_{eq}$), 3.97 (3H, s, 4′- or 5′-OCH₃), 3.99 (3H, s, 5′- or 4′-OCH₃), 4.20 (1H, m$_c$, 3-H), 5.48 (1H, m$_c$, 1-H), 6.75−7.21 (6H, m, arom H), 8.34 (1H, s, CHO);

EIMS (70 eV) m/z 489 (100) [M⁺], 474 (8) [M⁺ − CH₃], 405 (16) [M⁺ − C₅H₈O], 390 (75) [M⁺ − C₆H₁₁O]; HREIMS m/z 489.2520 (calcd for C₃₀H₃₅NO₅, 489.2515).

N-Formyl-8-O-lauroyldioncophylline A (12). As described above, 100 mg (0.247 mmol) of **12** was treated with 41 μL (30.0 mg, 0.296 mmol) of triethylamine, 70 μL (64.4 mg, 0.296 mmol) of lauroyl chloride, and a catalytic amount of DMAP to give **12** (109 mg, 75%) as colorless crystals: mp 27 °C; $[\alpha]^{20}_D$ +2.4° (c 0.50, CHCl₃); IR (KBr) ν_{max} 2957 (s, CH), 2822 (s, CH), 1700 (s, C=O, lauroyl), 1621 (m, C=O, formyl), 1583 (m, C=C), 1458 (m, CH₂), 1386 (m, CH₃), 1268 (m, C−O), 751 (w, CH) cm⁻¹; ¹H NMR (200 MHz) main rotational isomer with respect to the N-formyl bond δ 0.83−2.38 [23H, m, CO-(CH₂)₁₀CH₃], 1.33 (3H, d, J = 6.9 Hz, 3-CH₃), 1.39 (3H, d, J = 6.9 Hz, 1-CH₃), 2.20 (3H, s, 2′-CH₃), 2.81 (1H, dd, J = 15.2, 5.4 Hz, 4-H$_{ax}$), 3.27 (1H, dd, J = 16.5, 4.6 Hz, 4-H$_{eq}$), 3.96 (3H, s, 4′- or 5′-OCH₃), 3.98 (3H, s, 5′- or 4′-OCH₃), 4.20 (1H, m$_c$, 3-H), 5.45 (1H, q, J = 7.2 Hz, 1-H), 6.74−7.23 (6H, m, arom H), 8.35 (1H, s, CHO); EIMS (70 eV) m/z 587 (100) [M⁺], 405 (47) [M⁺ − C₁₂H₂₂O], 390 (65) [M⁺ − C₁₃H₂₅O], 376 (8) [M⁺ − C₁₃H₂₃O₂], 362 (8) [M⁺ − C₁₄H₂₅O₂]; HREIMS m/z 587.3593 (calcd for C₃₇H₄₉NO₅, 587.3611).

8-O-Benzoyl-N-formyldioncophylline A (13). According to the general procedure, 100 mg (0.247 mmol) of **2** was converted to **13** (80.6 mg, 64%) by treatment with 41 μL (30.0 mg, 0.296 mmol) of triethylamine, 34 μL (41.6 mg, 0.296 mmol) of benzoyl chloride, and a catalytic amount of DMAP. Compound **13** was obtained as colorless crystals: mp 183 °C; $[\alpha]^{20}_D$ +81.3° (c 0.50, CHCl₃); IR (KBr) ν_{max} 2950 (w, CH), 2905 (w, CH), 1723 (s, C=O, benzoyl), 1679 (s, C=O, formyl), 1667 (s, C=O, formyl), 1570 (m, C=C), 1381 (s, CH₃), 1245 (s, C−O), 749 (w, CH), 710 (w, CH) cm⁻¹; ¹H NMR (200 MHz) main rotational isomer with respect to the N-formyl bond δ 1.36 (3H, d, J = 6.6 Hz, 3-CH₃), 1.43 (3H, d, J = 6.7 Hz, 1-CH₃), 2.26 (3H, s, 2′-CH₃), 2.85 (1H, dd, J = 15.5, 5.2 Hz, 4-H$_{ax}$), 3.32 (1H, dd, J = 15.2, 5.5 Hz, 4-H$_{eq}$), 3.84 (3H, s, 4′- or 5′-OCH₃), 3.91 (3H, s, 5′- or 4′-OCH₃), 4.23 (1H, m$_c$, 3-H), 5.54 (1H, q, J = 6.3 Hz, 1-H), 6.63−8.12 (11H, m, arom H), 8.34 (1H, s, CHO); EIMS (70 eV) m/z 509 (100) [M⁺], 494 (14) [M⁺ − CH₃], 480 (3) [M⁺ − CHO], 404 (6) [M⁺ − C₇H₅O], 388 (10) [M⁺ − C₇H₅O₂], 360 (4) [M⁺ − C₈H₅O₃], 105 (49) [C₇H₅O⁺], 77 (10) [C₆H₅⁺]; HREIMS m/z 509.2202 (calcd for C₃₂H₃₁NO₅, 509.2202).

General Procedure for the Synthesis of 8-O-Alkyldioncophylline A Derivatives 14 and 15 from 8-O-Alkyl-N-formyldioncophylline A Derivatives 5 and 6. To a gently refluxing mixture of the corresponding 8-O-alkyl-N-formyldioncophylline A (**5 or 6**) in 10 mL MeOH, 1-mL portions of cold saturated methanolic HCl were added over a period of 24 h. After removal of the solvent, chromatography on Si gel (CH_2Cl_2-MeOH 9:1) afforded **14** and **15**, which were, after transformation into the corresponding hydrochlorides, recrystallized from MeOH.

8-O-Isopropyldioncophylline A (14). Following the above-mentioned general reaction procedure, 100 mg (0.223 mmol) of **5** was converted to **14** (89.5 mg, 88%), which was obtained as colorless needles: mp >350 °C dec; $[\alpha]^{20}_D$ +30.3° (c 0.50, CHCl₃); IR (KBr) ν_{max} 3340 (br, NH), 2943 (m, CH), 2916 (m, CH), 1593 (s, C=C), 1577 (s, C=C), 1380 (s, CH₃), 1250 (m, C−O), 1083 (s,

C−O), 744 (m, CH) cm^{-1}; ^{1}H NMR (200 MHz) δ 0.66 [3H, d, $J = 6.0$ Hz, CH(CH$_3$)CH_3], 1.02 [3H, d, $J = 6.0$ Hz, CH(CH_3)CH$_3$], 1.77 (3H, d, $J = 6.4$ Hz, 3-CH$_3$), 1.78 (3H, d, $J = 6.6$ Hz, 1-CH$_3$), 2.12 (3H, s, 2′-CH$_3$), 3.04 (1H, dd, $J = 16.8$, 4.4 Hz, 4-H$_{ax}$), 3.24 (1H, dd, $J = 17.2$, 11.2 Hz, 4-H$_{eq}$), 3.48 [3H, m$_c$, CH(CH$_3$)CH$_3$], 3.73 (1H, m$_c$, 3-H), 3.99 (3H, s, 4′- or 5′-OCH$_3$), 4.00 (3H, s, 5′- or 4′-OCH$_3$), 4.93 (1H, q, $J = 5.8$ Hz, 1-H), 6.69−7.32 (6H, m, arom H), 10.15 (1H, br s, NH); EIMS (70 eV) m/z 419 (22) [M$^+$], 404 (64) [M$^+$ − CH$_3$], 390 (67) [M$^+$ − C$_2$H$_5$], 362 (95) [M$^+$ − C$_4$H$_9$], 348 (100) [M$^+$ − C$_5$H$_{11}$]; HREIMS m/z 419.2453 (calcd for C$_{27}$H$_{33}$NO$_3$, 419.2460).

8-O-Benzyldioncophylline A (15). As described above, 100 mg (0.202 mmol) of **6** was converted to **15** (90.6 mg, 89%), which was obtained as colorless needles: mp 224 °C; [α]^{20}$_D$ −96.4° (c 0.25, CHCl$_3$); IR (KBr) ν_{max} 3405 (br, NH), 2942 (m, CH), 2917 (m, CH), 1582 (s, C=C), 1383 (s, CH$_3$), 1254 (s, C−O), 1204 (m, C−O), 750 (m, CH) cm^{-1}; ^{1}H NMR (200 MHz) δ 1.73 (3H, d, $J = 6.3$ Hz, 3-CH$_3$), 1.78 (3H, d, $J = 6.9$ Hz, 1-CH$_3$), 2.14 (3H, s, 2′-CH$_3$), 3.04 (1H, dd, $J = 17.2$, 4.5 Hz, 4-H$_{ax}$), 3.26 (1H, dd, $J = 17.3$, 10.5 Hz, 4-H$_{eq}$), 3.74 (1H, m$_c$, 3-H), 3.99 (3H, s, 4′- or 5′-OCH$_3$), 4.00 (3H, s, 5′- or 4′-OCH$_3$), 4.16 [1H, d, $J = 11.0$ Hz, C(H)H(C$_6$H$_5$)], 4.41 [1H, d, $J = 11.0$ Hz, C(H)H(C$_6$H$_5$)], 4.86 (1H, q, $J = 6.9$ Hz, 1-H), 6.74 (1H, s, 3′-H), 6.76−6.84 [2H, m, CH$_2$(C$_6$H$_5$)], 6.97 (1H, d, $J = 8.0$ Hz, 6′-H), 7.07 (1H, d, $J = 7.8$ Hz, 8′-H), 7.11−7.17 [3H, m, CH$_2$(C$_6$H$_5$)], 7.21 (1H, s, 6-H), 7.31 (1H, t, $J = 8.4$ Hz, 7′-H), 10.09 (1H, br s, NH); EIMS (70 eV) m/z 467 (24) [M$^+$], 452 (100) [M$^+$ − CH$_3$], 438 (8) [M$^+$ − C$_2$H$_5$], 375 (8) [M$^+$ − C$_7$H$_8$], 344 (36) [M$^+$ − C$_8$H$_{11}$O], 91 (10) [C$_7$H$_7^+$]; HREIMS m/z 467.2461 (calcd for C$_{31}$H$_{33}$NO$_3$, 467.2460).

General Procedure for the Synthesis of 8-O-Alkyldioncophylline A Derivatives 16−19 from N-Formyldioncophylline A (2). A suspension of **2**, 1.5 equiv of the corresponding alkyl halide, and 1.5 equiv Cs$_2$CO$_3$ in 20 mL Me$_2$CO was stirred at 20 °C for 48 h and subsequently filtered through Si gel (CH$_2$Cl$_2$). After removal of the solvent *in vacuo*, the residue was dissolved in 10 mL of MeOH, heated to reflux, and treated with 1-mL portions of cold saturated methanolic HCl over a period of 24 h. Chromatography on Si gel (CH$_2$Cl$_2$−MeOH 9:1) afforded **16**−**19**, which were recrystallized from MeOH, after conversion into hydrochloride salts.

8-O-(p-Bromobenzyl)dioncophylline A (16). As described above, 100 mg (0.247 mmol) of **2** was converted to **16** (128 mg, 89%) using 121 mg (0.371 mmol) of Cs$_2$CO$_3$ and 92.7 mg (0.371 mmol) of 4-bromobenzyl bromide. Compound **16** was obtained as colorless needles: mp 236 °C; [α]^{20}$_D$ +7.3° (c 0.50, CHCl$_3$); IR (KBr) ν_{max} 3382 (br, NH), 2917 (m, CH), 2818 (w, CH), 1570 (s, C=C), 1382 (s, CH$_3$), 1253 (m, C−O), 828 (m, CBr), 742 (m, CH) cm^{-1}; ^{1}H NMR (200 MHz) δ 1.73 (3H, d, $J = 6.1$ Hz, 3-CH$_3$), 1.81 (3H, d, $J = 6.7$ Hz, 1-CH$_3$), 2.11 (3H, s, 2′-CH$_3$), 3.05 (1H, dd, $J = 17.3$, 4.3 Hz, 4-H$_{ax}$), 3.24 (1H, dd, $J = 17.6$, 10.7 Hz, 4-H$_{eq}$), 3.76 (1H, m$_c$, 3-H), 3.99 (3H, s, 4′- or 5′-OCH$_3$), 3.99 (3H, s, 5′- or 4′-OCH$_3$), 4.09 [1H, d, $J = 12.4$ Hz, C(H)H(C$_6$H$_4$Br)], 4.41 [1H, d, $J = 11.8$ Hz, C(H)H(C$_6$H$_4$Br)], 4.89 (1H, q, $J = 6.9$ Hz, 1-H), 6.56−7.22 (9H, m, arom H), 7.29 (1H, t, $J = 8.6$ Hz, 7′-H), 10.08 (1H, br s, NH); EIMS (70 eV) m/z 547/545 (34/34) [M$^+$], 532/530 (100/97) [M$^+$ − CH$_3$], 361 (32) [M$^+$ − C$_8$H$_9$Br], 344 (53) [M$^+$ − C$_8$H$_{10}$BrO], 171/

169 (5/6) [C$_7$H$_6$Br$^+$]; HREIMS m/z 530.1328 (calcd for C$_{30}$H$_{27}$NBrO$_3$, 530.1331).

8-O-(p-Chlorobenzyl)dioncophylline A (17). According to the general reaction procedure, 100 mg (0.247 mmol) of **2** was treated with 121 mg (0.371 mmol) of Cs$_2$CO$_3$ and 59.7 mg (0.371 mmol) of 4-chlorobenzyl chloride and subsequently with methanolic HCl to give **17** (121 mg, 91%) as colorless needles: mp 225 °C; [α]^{20}$_D$ −22.8° (c 0.50, CHCl$_3$); IR (KBr) ν_{max} 3375 (br, NH), 2917 (m, CH), 2821 (w, CH), 1579 (s, C=C), 1381 (s, CH$_3$), 1252 (m, C−O), 802 (w, CCl), 749 (w, CH) cm^{-1}; ^{1}H NMR (200 MHz) δ 1.73 (3H, d, $J = 6.7$ Hz, 3-CH$_3$), 1.81 (3H, d, $J = 6.7$ Hz, 1-CH$_3$), 2.11 (3H, s, 2′-CH$_3$), 3.05 (1H, dd, $J = 17.3$, 4.6 Hz, 4-H$_{ax}$), 3.24 (1H, dd, $J = 17.3$, 10.9 Hz, 4-H$_{eq}$), 3.75 (1H, m$_c$, 3-H), 3.99 (3H, s, 4′- or 5′-OCH$_3$), 3.99 (3H, s, 5′- or 4′-OCH$_3$), 4.11 [1H, d, $J = 11.5$ Hz, C(H)H(C$_6$H$_4$Cl)], 4.41 [1H, d, $J = 11.3$ Hz, C(H)H(C$_6$H$_4$Cl)], 4.88 (1H, m$_c$, 1-H), 6.57−7.17 (9H, m, arom H), 7.29 (1H, t, $J = 8.6$ Hz, 7′-H), 10.08 (1H, br s, NH); EIMS (70 eV) m/z 503/501 (12/31) [M$^+$], 488/486 (39/100) [M$^+$ − CH$_3$], 376 (7) [M$^+$ − C$_7$H$_6$Cl], 361 (26) [M$^+$ − C$_8$H$_9$Cl], 344 (47) [M$^+$ − C$_8$H$_{10}$ClO], 127/125 (4/7) [C$_7$H$_6$Cl$^+$]; HREIMS m/z 501.2063 (calcd for C$_{31}$H$_{32}$NClO$_3$, 501.2071).

8-O-(p-Nitrobenzyl)dioncophylline A (18). According to the general procedure, 100 mg (0.247 mmol) of **2** was converted to **18** (122 mg, 90%), using 121 mg (0.371 mmol) of Cs$_2$CO$_3$ and 80.2 mg (0.371 mmol) of 4-nitrobenzyl bromide. Compound **18** was obtained as yellowish needles: mp 265 °C; [α]^{20}$_D$ −23.4° (c 0.50, CHCl$_3$); IR (KBr) ν_{max} 3380 (br, NH), 2920 (m, CH), 2823 (w, CH), 1579 (m, C=C), 1381 (m, CH$_3$), 1335 (s, NO$_2$), 1254 (m, C−O), 752 (m, CH), 736 (m, CH) cm^{-1}; ^{1}H NMR (250 MHz) δ 1.73 (3H, d, $J = 6.4$ Hz, 3-CH$_3$), 1.83 (3H, d, $J = 6.7$ Hz, 1-CH$_3$), 2.11 (3H, s, 2′-CH$_3$), 3.06 (1H, dd, $J = 17.7$, 4.6 Hz, 4-H$_{ax}$), 3.24 (1H, dd, $J = 17.7$, 11.6 Hz, 4-H$_{eq}$), 3.77 (1H, m$_c$, 3-H), 3.94 (3H, s, 4′- or 5′-OCH$_3$), 3.96 (3H, s, 5′- or 4′-OCH$_3$), 4.27 [1H, d, $J = 12.8$ Hz, C(H)H(C$_6$H$_4$NO$_2$)], 4.61 [1H, d, $J = 12.8$ Hz, C(H)H(C$_6$H$_4$NO$_2$)], 4.91 (1H, q, $J = 6.4$ Hz, 1-H), 6.76−7.95 (9H, m, arom H), 7.27 (1H, t, $J = 8.2$ Hz, 7′-H), 10.11 (1H, br s, NH); EIMS (70 eV) m/z 512 (36) [M$^+$], 497 (100) [M$^+$ − CH$_3$], 361 (34) [M$^+$ − C$_8$H$_9$NO$_2$], 344 (63) [M$^+$ − C$_8$H$_{10}$NO$_3$]; HREIMS m/z 512.2310 (calcd for C$_{31}$H$_{32}$N$_2$O$_5$, 512.2311).

8-O-[p-(Trifluoromethyl)benzyl]dioncophylline A (19). Compound **2** (100 mg, 0.247 mmol) was treated as described above with 121 mg (0.247 mmol) of Cs$_2$CO$_3$ and 57 μL (88.7 mg, 0.371 mmol) of α′-bromo-α,α,α-trifluoro-p-xylene and subsequently with methanolic HCl to give **19** (115 mg, 87%) as colorless needles: mp 219 °C; [α]^{20}$_D$ −17.7° (c 0.50, CHCl$_3$); IR (KBr) ν_{max} 3405 (br, NH), 2909 (m, CH), 2811 (w, CH), 1578 (m, C=C), 1381 (m, CH$_3$), 1316 (s, CF$_3$), 1252 (m, C−O), 818 (w, CF), 749 (w, CH) cm^{-1}; ^{1}H NMR (200 MHz) δ 1.71 (3H, d, $J = 6.3$ Hz, 3-CH$_3$), 1.82 (3H, d, $J = 6.6$ Hz, 1-CH$_3$), 2.11 (3H, s, 2′-CH$_3$), 3.05 (1H, dd, $J = 16.9$, 4.6 Hz, 4-H$_{ax}$), 3.23 (1H, dd, $J = 17.5$, 10.7 Hz, 4-H$_{eq}$), 3.76 (1H, m$_c$, 3-H), 3.96 (3H, s, 4′- or 5′-OCH$_3$), 3.98 (3H, s, 5′- or 4′-OCH$_3$), 4.21 [1H, d, $J = 11.5$ Hz, C(H)H(C$_6$H$_4$CF$_3$)], 4.54 [1H, d, $J = 11.0$ Hz, C(H)H(C$_6$H$_4$CF$_3$)], 4.91 (1H, q, $J = 7.2$ Hz, 1-H), 6.67−7.38 (9H, m, arom H), 7.33 (1H, t, $J = 8.6$ Hz, 7′-H), 10.07 (1H, br s, NH); EIMS (70 eV) m/z 535 (35) [M$^+$], 520 (100) [M$^+$ − CH$_3$], 361 (22) [M$^+$ − C$_8$H$_6$F$_3$], 344 (41) [M$^+$ − C$_8$H$_7$F$_3$O], 159 (4)

$[C_8H_6F_3^+]$; HREIMS m/z 535.2332 (calcd for $C_{32}H_{32}$-NF_3O_3, 535.2334).

5-Bromodioncophylline A (20). A solution of 100 mg (0.265 mmol) of dioncophylline A (**1**), 52.0 mg (0.291) of *N*-bromosuccinimide (NBS), and a catalytic amount of α,α′-azoisobutyronitrile (AIBN) in 30 mL cyclohexane−CHCl$_3$ (20:1) was stirred at 0° for 4 days. After chromatography on Si gel (CH$_2$Cl$_2$−MeOH 9:1), **20** was converted into its hydrochloride and recrystallized from MeOH (98.0 mg, 75%) to give colorless needles: mp 228 °C (lit.[20] mp 225 °C); $[\alpha]^{20}_D$ −63.4° (*c* 0.25, CHCl$_3$) [ref.[20] $[\alpha]^{20}_D$ −69.9° (*c* 0.72, CHCl$_3$)]; spectroscopic and chromatographic data are identical with authentic **20** as prepared by another route.[20]

Experiments with Insects. Larvae of *S. littoralis* were taken from a laboratory colony reared on artificial diet under controlled conditions as described previously.[21] Chronic feeding bioassays were conducted with neonate larvae that were kept on diet spiked with different concentrations of the compounds studied. Known doses of each compound dissolved in appropriate amounts of MeOH were added to equal portions of freeze-dried diet (lacking agar) to obtain an even coating of the dry diet powder. After complete evaporation of the carrier solvent (24 h) appropiate amounts of H$_2$O and agar were added to obtain the complete artificial diet. Control diet was treated with MeOH only. Groups of 20 neonate larvae were subsequently released on each cube of treated diet ($n = 20$ for each dose). After 6 days, survival and weight of the surviving larvae were monitored.

Initially each of the studied alkaloids (**1**−**15**, **20**) was tested at the same range of concentrations (25, 50, 100, 250, 500, 750 μg/g fresh wt). In a second experiment, the more active compounds (**15**−**19**) were studied for growth inhibitory effects under comparable conditions but employing lower concentrations of compounds (5, 10, 20, 40, 60, 80, and 100 μg/g fresh wt). From the dose-response curves obtained in the experiments, the effective concentration that inhibited growth by 50% (EC$_{50}$) was determined from the regression-growth curve.

References and Notes

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