12. Neurotoxic Sesquiterpenoids from the Yellow Star Thistle *Centaurea solstitialis* L. (Asteraceae)

by **Ying Wang**[a]), **Matthias Hamburger**[a]), **Celine H. K. Cheng**[b]), **Brenda Costall**[b]), **Robert J. Naylor**[b]), **Peter Jenner**[c]), and **Kurt Hostettmann**[a])*

[a]) Institut de Pharmacognosie et Phytochimie, Université de Lausanne, 2, rue Vuillermet, CH–1005 Lausanne
[b]) Postgraduate Studies in Pharmacology, The School of Pharmacy, University of Bradford, Bradford, West Yorkshire BD7 1DP, United Kingdom
[c]) Parkinson Disease Society Experimental Research Laboratories, Pharmacology Group, Biomedical Sciences Division, King's College, Manresa Road, London SW3 6LX, United Kingdom

(4.X.90)

Ingestion of yellow star thistle (*Centaurea solstitialis* L.) by horses produces parkinsonism due to nigro-pallidal degeneration. The toxin responsible has not been identified so far. A CH_2Cl_2 extract from the aerial parts of *C. solstitialis* exhibited significant neurotoxicity against primary neuronal cultures of foetal rat brain. Activity-guided fractionation yielded the known sesquiterpene lactones solstitialin A (**1**), 13-*O*-acetylsolstitialin A (**3**), cynaropicrin (**4**), and the hitherto unknown 3-*O*-acetylsolstitialin A (**2**). In the bioassay with rat foetal full cell culture, **3** and **4** were toxic in a concentration-dependent manner and may be responsible for the ability of the plant to cause neurodegenerative changes in the brain of horses.

Introduction. – Yellow star thistle (*Centaurea solstitialis* L., Asteraceae) is a weed that has its origin in eastern Eurasia. It is a pioneering species that is highly invasive and has the ability to spread rapidly and colonize on disturbed soils. The yellow star thistle is now naturalized in most of the temperate areas of the world. It has become particularly menacing in the western USA (California, Oregon, Idaho) where large areas of waste-land, rangeland, and cultivated fields have been invaded by this weed [1].

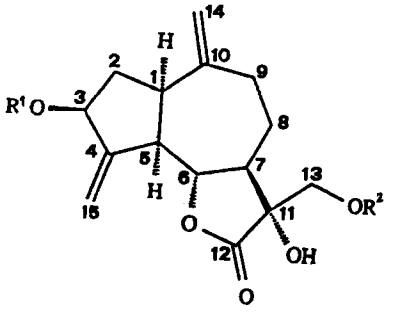
In 1954, a specific neurological disease of horses occurring in central and northern California, locally known as 'chewing disease' or 'yellow star thistle poisoning', was experimentally linked to the ingestion of large amounts of *C. solstitialis* [2]. Horses feeding on the thistle develop within 1 to 3 months a syndrome initially characterized by immobility of the facial musculature, idle chewing and tongue flicking, and impaired eating and drinking, followed by hypokinesia and a lack of reactivity which persists until death. Neuropathological examination of the brains revealed bilateral necrosis of the anterior globus pallidus and zona reticulata of substantia nigra. Therefore, the disease has been named nigro-pallidal encephalomalacia [3]. The syndrome can be reproduced in horses fed experimentally on the thistle but is not observed in cattle or sheep which graze on the thistle. The most likely explanation for the neurological changes observed and the neuropathological changes occurring in brain is that yellow star thistle contains substances which on oral ingestion are neurotoxic to the horse.

The current interest in neurotoxins stems from the fact that neurodegenerative diseases of the basal ganglia produce a variety of abnormal movement disorders such as *Parkinson*'s disease and *Huntington*'s chorea. The cause of these illnesses is not known,

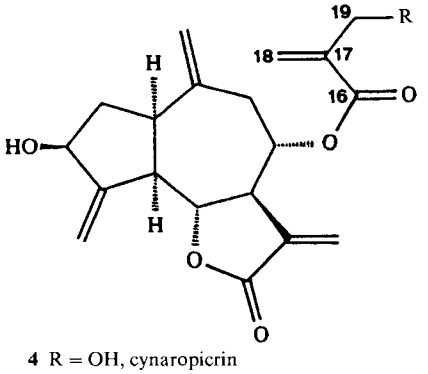
but there has been considerable interest in the role that might be played by neurotoxins. *E.g.*, the synthetic toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) selectively destroys nigral dopamine containing cells and produces parkinsonism in man and other primate species [4]. Natural toxins may also induce neurodegenerative diseases. Thus, the non-protein amino acids BMAA (= 3-(methylamino)-L-alanine) and BOAA (= 3-(oxalylamino)-L-alanine) contained in the false sago palm and chickling pea, respectively, give rise to motor neurone disease and lathyrism.

Other potentially useful neurotoxins may be contained within the yellow star thistle. Previous phytochemical investigations on this species revealed the presence of alkaloids [5], triterpenoids [6], polyacetylenes [7], flavonoids [8], and a series of sesquiterpene lactones [9–14]. Polyacetylenic compounds and sesquiterpene lactones are well known to possess a broad spectrum of biological activities, but the compounds responsible for the neurotoxic effect on horses have not been identified. While this work was in progress, the sesquiterpene lactone repin isolated from the closely related species *C. repens* and previously also reported from *C. solstitialis* was shown to possess high toxicity towards chick embryo sensory neurons [15]. As a continuation of our previous studies on biologically active compounds from Asteraceae [16], we investigated *C. solstitialis* in an attempt to identify constituents which might be the causative agents of equine nigro-pallidal encephalomalacia.

Results. – Rat foetal brain neuronal cells in culture were used as a bioassay suitable for activity-guided fractionation of the extract, since the neuropathological studies on horses had revealed degenerative lesions in the substantia nigra and globus pallidus. Preliminary assays carried out with the petroleum ether, $CHCl_3$, and MeOH extracts showed that the $CHCl_3$ extract was significantly toxic (30–40% dead cells) at a concentration of 2.5 μg/ml to cell cultures derived from the substantia nigra, frontal cortex, striatum, and the raphe nuclei, whereas the petroleum ether and MeOH extracts caused no significant cell death at the same concentration. Thus, for isolation of the neurotoxic constituents, the plant material was directly extracted with CH_2Cl_2. This extract was submitted to column chromatography on silica gel to afford ten fractions. *Fractions 5–8* exhibited significant toxicity (20–50% cell death at 2.5 μg/ml) against rat mesencephalic full cell culture, whereas other fractions were not active at the highest concentration tested. Full details of the results from the bioassays will be reported elsewhere.



1 $R^1 = H$, $R^2 = H$, solstitialin A
2 $R^1 = Ac$, $R^2 = H$
3 $R^1 = H$, $R^2 = Ac$



4 R = OH, cynaropicrin
5 R = H, aguerin B

Table 1. *^{1}H-NMR Data of Compounds* **1**–**4**. δ in ppm rel. to TMS (= 0 ppm), J in Hz.

H-Atom	**1**[a])	**2**[a])	**3**[b])	**4**[a])[c])
H–C(1)	2.87 (*ddddd*, J = 8.1, 8.1, 7.0, 1.5, 1.5)	2.93 (*ddddd*, J = 8.3, 8.3, 6.8, 1.5, 1.5)	2.89 (*ddddd*, J = 7.7, 7.7, 7.7, 1.5, 1.5)	2.97 (*ddddd*, J = 11.2, 9.4, 7.7, 1.5, 1.5)
H_a–C(2)	1.70 (*ddd*, J = 13.1, 8.1, 8.1)	1.81 (*ddd*, J = 13.9, 6.8, 6.8)	1.73 (*ddd*, J = 13.5, 7.7, 7.7)	1.73 (*ddd*, J = 13.5, 11.2, 8.8)
H_b–C(2)	2.31 (*ddd*, J = 13.1, 8.1, 7.0)	2.46 (*ddd*, J = 13.9, 8.3, 8.3)	2.35 (*ddd*, J = 13.5, 7.7, 7.7)	2.18 (*ddd*, J = 13.5, 7.7, 7.7)
H–C(3)	4.50 (*dddd*, J = 8.1, 8.1, 2.0, 2.0)	5.53 (*dddd*, J = 8.3, 6.8, 2.0, 2.0)	4.55 (*dddd*, J = 7.7, 7.7, 1.5, 1.5)	4.52 (*dddd*, J = 8.8, 7.7, 2.0, 2.0)
H–C(5)	2.78 (*dddd*, J = 9.4, 8.1, 2.0, 2.0)	2.82 (*dddd*, J = 10.0, 8.3, 2.0, 2.0)	2.83 (*dddd*, J = 9.7, 7.7, 1.5, 1.5)	2.84 (*dddd*, J = 10.2, 9.4, 2.0, 2.0)
H–C(6)	4.24 (*dd*, J = 9.4, 9.4)	4.21 (*dd*, J = 10.0, 10.0)	4.16 (*dd*, J = 9.7, 9.7)	4.29 (*dd*, J = 10.2, 9.0)
H–C(7)	2.36 (*ddd*, J = 13.0, 9.4, 4.5)	2.38 (*ddd*, J = 13.4, 10.0, 3.3)	2.45 (*ddd*, J = 12.3, 9.7, 4.4)	3.22 (*dddd*, J = 9.0, 9.0, 3.0, 3.0)
H_a–C(8)	1.61 (*dddd*, J = 13.0, 13.0, 11.0, 4.5)	1.60 (*dddd*, J = 13.4, 13.4, 13.4, 3.3)	1.38 (*dddd*, J = 12.3, 12.3, 12.3, 4.4)	5.18 (*ddd*, J = 9.0, 5.0, 3.5)
H_b–C(8)	2.07 (*dddd*, J = 13.0, 4.5, 4.5, 4.5)	2.10 (*dddd*, J = 10.0, 3.3, 3.3, 3.3)	2.14 (*dddd*, J = 12.3, 3.4, 3.4, 3.4)	–
H_a–C(9)	2.58 (*ddd*, J = 13.0, 4.5, 4.5)	2.53 (*ddd*, J = 13.4, 13.4, 3.3)	2.57 (*ddd*, J = 12.3, 4.4, 4.4)	2.72 (*dd*, J = 14.6, 5.0)
H_b–C(9)	1.99 (*ddd*, J = 13.0, 11.0, 4.5)	2.13 (*ddd*, J = 13.4, 13.4, 3.3)	1.98 (*ddd*, J = 12.3, 12.3, 4.4)	2.40 (*dd*, J = 14.6, 3.5)
H_a–C(13)	3.70 (*s*)	3.70 (*s*)	4.16 (*d*, J = 11.6)	5.66 (*d*, J = 3.0)
H_b–C(13)	3.70 (*s*)	3.70 (*s*)	4.13 (*d*, J = 11.6)	6.21 (*d*, J = 3.0)
H_a–C(14)	4.94 (br. *s*)	4.90 (br. *s*)	4.95 (br. *s*)	4.93 (*d*, J = 1.5)
H_b–C(14)	4.94 (br. *s*)	4.94 (br. *s*)	4.97 (br. *s*)	5.15 (*d*, J = 1.5)
H_a–C(15)	5.31 (*dd*, J = 2.0, 2.0)	5.27 (*dd*, J = 2.0, 2.0)	5.33 (*dd*, J = 1.5, 1.5)	5.37 (*d*, J = 2.0)
H_b–C(15)	5.34 (*dd*, J = 2.0, 2.0)	5.39 (*dd*, J = 2.0, 2.0)	5.36 (*dd*, J = 1.5, 1.5)	5.47 (*d*, J = 2.0)
H_a–C(18)	–	–	–	5.96 (*d*, J = 1.1)
H_b–C(18)	–	–	–	6.34 (*d*, J = 1.1)
H–C(19)	–	–	–	4.35 (br. *s*)[c])
CH_3CO	–	2.11 (*s*)	2.13 (*s*)	–

[a]) In $CDCl_3/CD_3OD$ 9:1.

[b]) In $CDCl_3$.

[c]) The chemical shifts of aguerin B (**5**) in $CDCl_3$ were virtually identical, with the exception of $CH_2(19)$ ($CH_3(19)$ of **5** at 1.94 ppm) [19].

Due to the small quantities of *Fractions 5* and *8*, further isolation was carried out only on active *Fractions 6* and *7*. Compound **3** was obtained from *Fraction 6*, while *Fraction 7* afforded **2** and **4** by chromatography on *Sephadex LH-20*, silica gel, and *Diol*. In addition, compound **1** was obtained from *Fraction 9* by gel filtration on *Sephadex LH-20* (see *Exper. Part*). Among the four compounds isolated, **3** and **4** exhibited neurotoxic activity in the test system using rat mesencephalic full culture. These compounds caused a concentration-dependent reduction in the percentage of live cells. The IC_{50} values (micromolar concentration to cause 50% cell death) were 3.6 ± 0.31 and 3.0 ± 0.1 (mean ± s.e.m., $n = 4$) for compounds **3** and **4**, respectively.

Compound **1** was identified as the guaianolide solstitialin A, a sesquiterpene lactone that has already been reported from *C. solstitialis* [9] [10]. Originally, its structure was established by X-ray diffraction analysis of the 4-bromobenzoate [10], and its NMR spectral data have been reported [13]. More extensive ^{1}H- and ^{13}C-NMR measurements (*Tables 1* and *2*) have now been performed (homonuclear spin decoupling, DQ PSCOSY, NOE difference measurements, and DEPT spectra).

Table 2. *^{13}C-NMR Data of Compounds* **1**–**4**. δ in ppm rel. to $CDCl_3$ (77.0 ppm).

C-Atom	1[a])	2[a])	3[b])	4[a])	C-Atom	1[a])	2[a])	3[b])	4[a])
C(1)	42.80	44.09	42.94	44.93	C(12)	179.03	179.73	176.52	169.61
C(2)	38.02	36.61	38.27	38.57	C(13)	62.93	63.50	64.13	125.82
C(3)	72.70	75.40	73.32	74.06	C(14)	113.10	113.60	113.62	112.92
C(4)	152.33	149.14	154.45	151.71	C(15)	110.43	113.45	111.31	117.83
C(5)	52.22	52.43	52.47	50.99	C(16)	–	–	–	165.24
C(6)	82.13	82.75	81.97	78.65	C(17)	–	–	–	139.49
C(7)	49.87	51.13	49.63	47.24	C(18)	–	–	–	122.58
C(8)	26.22	26.73	26.86	78.65	C(19)	–	–	–	72.88
C(9)	35.57	36.33	35.90	36.90	CH_3CO	–	171.88	170.44	–
C(10)	148.66	148.61	148.62	141.64	CH_3CO	–	21.30	20.57	–
C(11)	[c])	[c])	75.52	137.23					

[a]) In $CDCl_3/CD_3OD$ 9:1. [b]) In $CDCl_3$. [c]) Overlapped with the signal of $CDCl_3$.

Based on NMR and MS data, the minor sesquiterpenoid **2** was identified as 3-*O*-acetylsolstitialin A, a new natural compound. From DCI-MS and ^{13}C-NMR data, it was readily apparent that the major neurotoxic compound **3** was an isomer of 3-*O*-acetylsolstitialin A (**2**); it was identified as 13-*O*-acetylsolstitialin A, a sesquiterpene lactone that has already been isolated in the course of a phytochemical investigation of *C. solstitialis* [13].

The DCI-MS of **2** shows quasimolecular ions at *m/z* 340 ($[M + NH_4]^+$) and 323 ($[M + H]^+$) together with prominent fragment ions at *m/z* 280 and 263, resulting from an elimination of an Ac moiety. In the ^{1}H-NMR spectrum, the signal attributable to H–C(3) appears upfield by 1 ppm compared to parent **1**, while the remaining signals are virtually unaffected (see *Table 1*). The structure of **2** is corroborated by the ^{13}C-NMR data: Compared to **1**, the signal of C(3) (75.40 ppm) is shifted downfield by 2.7 ppm, whereas the signals of the adjacent C(2) (36.61 ppm) and C(4) (149.14 ppm) appear at higher field (+1.5 and 3.2 ppm, resp.).

DEPT multiplicities of **3** suggest a guaianolide skeleton [17]. Four signals attributable to two exocyclic double bonds (154.45 and 111.31 ppm; 148.62 and 113.62 ppm), four O-bearing sp^3 C-atoms (64.13, 73.32, 75.52, and 81.97 ppm), and an Ac group (170.48 and 20.57 ppm) indicate that **3** is an acetyl derivative of **1**. The DCI-MS confirms this. The ^{1}H-NMR signals of CH_2(13) (4.13 and 4.16 ppm) appear 0.43 ppm downfield from the corresponding signal in parent compound **1**. Compared to **1** and **2**, δ(C(13)) of **3** is slightly shifted to lower field (0.6 – 1.2 ppm), while the signal of vicinal C(11) appears upfield (*ca.* 2 ppm); (overlap of the C(11) signal of **1** and **2** with the solvent signal precludes its accurate localization).

In order to get first clues for the understanding of the apparently selective *in vitro* neurotoxic activity of **3** (parent compound **1** and positional isomer **2** were both inactive in the assay when tested at 25 μg/ml), the solution conformation of **1**–**3** was investigated by a detailed analysis of ^{1}H-NMR chemical shifts and vicinal coupling constants, and corroborated, in the case of **3**, by extensive NOE difference measurements (see the *Fig.* and

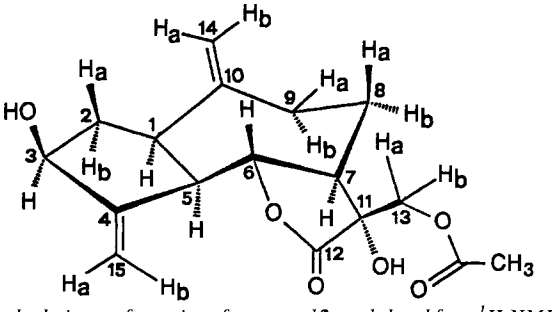


Figure. *Preferred solution conformation of compound* **3**, *as deduced from* ^{1}*H-NMR data* (see text)

Exper. Part). However, it appears that the preferred solution conformation of the tricyclic skeleton of **1**–**3** is the same; it corresponds to the solid conformation of the guaianolide skeleton in solstitialin A (**1**), as established by X-ray analysis [10]. But the conformation of the side chain at C(11) of **3** seems to adopt a fixed conformation which can be explained by a H-bond between the acetyl carbonyl and the tertiary OH group at C(11). Thus, in contrast to **1** and **2**, the CH_2(13) signals of **3** are not equivalent, and NOE difference measurements reveal a spatial vicinity of both H_a–C(13) and H_b–C(13) and H_a–C(8).

The second neurotoxic compound **4** was identified as cynaropicrin, a sesquiterpene lactone that has been first isolated from the artichoke *Cynara scolymus* [18]. Its structure was confirmed by spectroscopic means (see *Exper. Part*) and comparison with known ^{1}H-NMR data and that of aguerin B (**5**; see *Table 1*) [19].

Discussion. – Among the compounds isolated from yellow star thistle, 13-*O*-acetyl-solstitialin A (**3**) and cynaropicrin (**4**) exhibit neurotoxic activity against cultured rat foetal brain cells in a concentration-dependent manner. Solstitialin A (**1**) and 3-*O*-acetyl-solstitialin A (**2**) did not show any significant toxicity at the highest concentration tested. The comparison of the structural features of **1**–**3** is of particular interest, since **2** and **3** are both monoacetates of solstitialin A (**1**). Among the three compounds, only the 13-*O*-acetyl derivative **3** showed neurotoxicity. The differences in lipophilicity may be invoked as a reason for the absence or presence of toxicity. With three OH groups in the molecule, the inactive parent compound **1** is more polar than the acetyl derivatives **2** and **3**, which both have only two OH groups. Although bearing the same functional groups as inactive **2**, an intramolecular H-bond between OH–C(11) and the acetyl carbonyl group in neurotoxic **3**, as suggested by the ^{1}H-NMR spectra, would increase its lipophilicity. Another structural element may be responsible for the toxicity of cynaropicrin (**4**). This compound features, like numerous sesquiterpene lactones, an α-methylidene-γ-lactone moiety susceptible to undergo a *Michael*-type addition with biological nucleophiles [20]. Compounds bearing this structural element are generally cytotoxic *in vitro* [21]. Cynaropicrin, *e.g.*, has an ID_{50} of 5 μg/ml against HeLa cells [22]. We are currently

proceeding to a large-scale isolation of neurotoxic constituents of the yellow star thistle in view of *in vivo* assays and for structure-activity studies.

Financial support by the *Swiss National Science Foundation* is gratefully acknowledged. We thank Prof. *C. Andary*, Université de Montpellier, France, for providing the plant material.

Experimental Part

General. TLC: silica gel *60* F_{254} Al sheets (*Merck*); *Diol*-precoated HPTLC plates (*Merck*); solvent systems employed for silica gel: petroleum ether/AcOEt 1:1, $CHCl_3$/MeOH 95:5; for Diol: $CHCl_3$/MeOH 50:1, hexane/AcOEt 1:1; detection at 254 and 366 nm and with *Godin* reagent [23]. Column chromatography: silica gel (40–63 μm; *Merck*) and *Sephadex LH-20* (*Pharmacia*). Low-pressure liquid chromatography (LPLC): *Lobar LiChroprep Diol* (40–63 μm; 27 × 2.5 cm i.d.; *Merck*), equipped with a *Duramatic 80* pump (*Chemie und Filter*, Regensdorf); flow rate, 1 ml/min. Purity of compounds was checked by HPLC with a *Spectra-Physics-8700* pump (San José, USA) and a photodiode array detector *HP-1040A*, coupled with a *HP-85* personal computer and a *HP-7470A* plotter (*Hewlett Packard*). HPLC columns: *LiChrosorb RP-8* (7 μm; 4 × 250 mm i.d., *Knauer*). ^{1}H- and ^{13}C-NMR: *Varian VXR 200* at 200.06 MHz and 50.30 MHz, resp., in $CDCl_3$ or $CHCl_3$/MeOH 9:1; ^{1}H-NMR with 0.1 Hz digital resolution. NOE difference measurements were performed according to [24].

Plant Material. Centaurea solstitialis L. (aerial parts) was collected in July 1987 in Saint-Jean de Cucule, near Montpellier, France. A voucher specimen is deposited at the Institute of Pharmacognosy and Phytochemistry, School of Pharmacy, University of Lausanne.

Extraction and Separation. For preliminary biological testing, an aliquot of ground plant material was extracted at r.t. successively with petroleum ether, $CHCl_3$, and MeOH. Since neurotoxic activity was concentrated in the $CHCl_3$ extract, the remaining plant material (180 g) was extracted directly with CH_2Cl_2. This extract (5.3 g) was subjected to column chromatography (silica gel, $CHCl_3$/MeOH 98:2 → 90:10). A total of 10 fractions were collected. The neurotoxic *Fr. 5–8* were each chromatographed on *Sephadex LH-20* with CH_2Cl_2/MeOH 1:1 to remove chlorophyll. Compound **3** (80 mg) was obtained from *Fr. 6* by further chromatography (silica gel, $CHCl_3$/MeOH 9:1). From *Fr. 7*, **4** (26 mg) was also isolated by chromatography (first silica gel, $CHCl_3$/MeOH 95:5; then *Lobar Diol*, heptane/AcOEt/CF_3COOH 50:50:0.1). Further purification of remaining fractions were performed on a *Lobar Diol* column (hexane/$CHCl_3$/MeOH 100:90:10 and $CHCl_3$/MeOH 99:1) to afford **2** (10 mg). Compound **1** (9 mg) was obtained from *Fr. 9* by gel filtration (*Sephadex LH-20*, CH_2Cl_2/MeOH 1:1).

Preparation of Rat Foetal Full Cell Culture. Sprague-Dawley rat foetuses were taken at 15 days gestation. The brains were removed and the areas containing the mesencephalon to include the substantia nigra or raphe nucleus, frontal cortex, and striatum were dissected out in cold phosphate-buffer soln. (*Flow Laboratory*). The tissue was washed once with serum-supplement medium (= SSM) containing 2% of *Ultraser G* (*Gibco*, U.K.; equivalent to 10% foetal calf serum), 2 mM L-glutamine (*Sigma*), 100 units/ml of penicillin, and 100 μg/ml of streptomycin (*Sigma*) in equal parts of *Dulbecco*'s modification of *Eagle*'s medium and Ham F12 soln. (both from *Flow Laboratory*) and then mechanically disrupted by gently pipetting with a fire-narrowed *Pasteur* pipette in SSM until it was dissociated. The cell suspension was allowed to settle for *ca.* 3–5 min to remove any undissociated tissue and adjusted to 2×10^5 cells per ml with SSM. Then, 0.15 ml of the cell suspension was plated in each of 96 *Corning* cell wells which were pre-coated with poly-D-lysine (*Sigma*) and incubated at 37°, 100% humidity, and 5% CO_2. After 24 h of initial incubation, the medium was replaced with fresh medium containing the testing compound, and the culture was incubated for further 48 h. After the drug incubation period, medium was discarded. Live and dead cells were identified using ethidium bromide/acridine orange staining and fluorescence microscopy. For initial studies, the crude petroleum ether, $CHCl_3$, and MeOH extracts were dissolved in CH_2Cl_2, MeOH, and H_2O to give an initial soln. of 5 mg/ml. For further testing, all fractions and pure compounds were dissolved in DMSO to give an initial concentration of 5 mg/ml. Significant reductions compared to control were calculated with ANOVA followed by *Dunnett*'s *t* test. The results of bioassays are expressed as a percentage of live cells.

Solstitialin A (= *(3R*,3aR*,6aR*,8S*,9aR*,9bR*)-Perhydro-3,8-dihydroxy-3-(hydroxymethyl)-6,9-di(methylidene)azuleno[4,5-b]furan-2-one*; **1**). Colorless solid. M.p. 202–204°. TLC (SiO_2, $CHCl_3$/MeOH 9:1): R_f 0.20. $[\alpha]_D = +61$ ($c = 0.75$, $CHCl_3$/MeOH 1:1). ^{1}H-NMR: *Table 1*. ^{13}C-NMR: *Table 2*. DCI-MS (NH_3, pos.-ion mode): 298 ($[M(C_{15}H_{20}O_5) + NH_4]^+$), 281 ($[M + H]^+$), 280 ($[M]^+$), 263 ($[M + H - 18]^+$), 245 ($[M + H - 36]^+$).

3-O-Acetylsolstitialin A (**2**). Colorless solid. M.p. 163–165°. TLC (*Diol*, $CHCl_3$/MeOH 99:1): R_f 0.15. $[\alpha]_D = +69$ ($c = 0.33$, $CHCl_3$/MeOH 1:1). ^{1}H-NMR: *Table 1*. ^{13}C-NMR: *Table 2*. DCI-MS (NH_3, pos.-ion mode): 340 ($[M + NH_4]^+$), 323 ($[M + H]^+$), 280 ($[M + NH_4 - 60]^+$), 263 ($[M + H - 60]^+$), 245 ($[M + H - 78]^+$).

13-O-Acetylsolstitialin A (**3**). Colorless viscous oil. TLC (SiO_2, $CHCl_3$/MeOH 95:5): R_f 0.23. $[\alpha]_D = +57$ ($c = 0.50$, $CHCl_3$/MeOH 1:1). ^{1}H-NMR: *Table 1*. NOE: H–C(1) + H–C(5)/H–C(13), H–C(6), H–C(7), H_b–C(9), H_a–C(14), H_a–C(2)/H_b–C(2), H–C(6), H_b–C(14); H–C(3)/H–C(1), H_b–C(2), H–C(5), H_a–C(15); H–C(6) + CH_2(13)/H–C(1), H–C(5), H_b–C(15); H_b–C(8)H_a–C(8); H_a–C(8)/H_b–C(8), H_a–C(13), H_b–C(13); H_b–C(9)/H_a–C(9), H_a–C(14); H_a–C(14)/H_b–C(9); H_b–C(14)/H_a–C(2); H_b–C(15)/H_a–C(15), H–C(16). ^{13}C-NMR: *Table 2*. DCI-MS (NH_3, pos.-ion mode): 340 ($[M + NH_4]^+$), 323 ($[M + H]^+$), 322 ($[M]^+$), 305 ($[M + H - 18]^+$), 287 ($[M + H - 36]^+$), 280 ($[M + NH_4 - 60]^+$), 263 ($[M + H - 60]^+$), 245 ($[M + H - 78]^+$).

Cynaropicrin (= *(3aR*,4S*,6aR*,8S*,9aR*,9bR*)-Perhydro-8-hydroxy-3,6,9-tri(methylidene)-2-oxoazuleno[4,5-b]furan-4-yl 2-(Hydroxymethyl)prop-2-enoate*; **4**). Colorless viscous oil. TLC (SiO_2, toluene/MeOH 4:1): R_f 0.21. $[\alpha]_D = +57$ ($c = 0.50$, $CHCl_3$/MeOH 1:1). UV (MeOH): 247. ^{1}H-NMR: *Table 1*. ^{13}C-NMR: *Table 2*. DCI-MS (pos.-ion mode): 364 ($[M + NH_4]^+$), 347 ($[M + H]^+$), 263, 245 ($[M + H - C_4H_6O_3$ (102)$]^+$), 227 ($[M + H - C_4H_8O_4$ (120)$]^+$).

REFERENCES

[1] D. M. Maddox, A. Mayfield, N. H. Poritz, *Weed Sci.* **1985,** *33,* 315.

[2] D. R. Cordy, *J. Neuropath. Exp. Neurol.* **1954,** *13,* 330.

[3] D. R. Cordy, in 'Effects of Poisonous Plants on Livestock', Eds. R. F. Keeler, K. R. Van Kampen, and L. F. James, Academic Press, New York, 1978, pp. 327–336.

[4] J. A. Javitch, S. H. Snyder, *Eur. J. Pharmacol.* **1985,** *106,* 455; I. J. Kopin, S. P. Markey, *Ann. Rev. Neurosci.* **1988,** *11,* 81.

[5] G. M. Mamedov, *Apetechn. Delo.* **1956,** *5,* 57.

[6] J. M. Cassady, G. C. Hokanson, *Phytochemistry* **1978,** *17,* 324; K. L. Stevens, S. C. Witt, *J. Liq. Chromatogr.* **1989,** *12,* 1203.

[7] F. Bohlmann, K. M. Rode, C. Zdero, *Chem. Ber.* **1966,** *99,* 3544; J. L. Masso, M. N. Bertran, T. Adzet, *Plant Med. Phytother.* **1979,** *13,* 41; R. G. Buttery, D. M. Maddox, D. M. Light, L. C. Ling, *J. Agric. Food. Chem.* **1986,** *34,* 786; K. L. Stevens, S. C. Witt, *J. Liq. Chromatogr.* **1989,** *12,* 1203; R. G. Binder, C. E. Turner, R. A. Flath, *J. Agric. Food. Chem.* **1990,** *38,* 764.

[8] K. Kamanzi, J. Raynaud, *Plant Med. Phytother.* **1976,** *10,* 78; K. Kamanzi, J. Raynaud, B. Voirin, *Pharmazie* **1983,** *38,* 494; K. Kamanzi, B. Voirin, J. Raynaud, *Plant Med. Phytother.* **1983,** *17,* 52; K. Kamanzi, J. Raynaud, B. Voirin, *ibid.* **1983,** *17,* 57.

[9] N. Zarghami, F. E. Heinz, *Chem. Ind. (London)* **1969,** 1556.

[10] W. E. Thiessen, *Chem. Ind. (London)* **1969,** 460; W. E. Thiessen, H. Hope, *Acta Crystallogr., Sect. B* **1970,** *26,* 554.

[11] M. N. Mukhametzhanov, A. I. Shreter, D. A. Pakala, *Khim. Prir. Soedin.* **1969,** *5,* 590.

[12] J. M. Cassady, D. Abramson, P. Cowall, C. J. Chang, J. L. McLaughlin, Y. Aynechi, *J. Nat. Prod.* **1979,** *42,* 427.

[13] G. B. Merrill, K. L. Stevens, *Phytochemistry* **1985,** *24,* 2013.

[14] J. Jakupovic, Y. Jia, V. P. Pathak, F. Bohlmann, R. M. King, *Planta Med.* **1986,** *52,* 399.

[15] K. L. Stevens, R. J. Riopelle, R. Y. Wong, *J. Nat. Prod.* **1990,** *53,* 218.

[16] Y. Wang, M. Hamburger, J. Guého, K. Hostettmann, *Phytochemistry,* **1989,** *28,* 2323; Y. Wang, M. Toyota, F. Krause, M. Hamburger, K. Hostettmann, *ibid.* **1990,** *29,* 3101.

[17] N. H. Fischer, E. J. Olivier, H. D. Fischer, in 'Progress in the Chemistry of Organic Natural Products', Eds. W. Herz, H. Grisebach, and G. W. Kirby, Springer-Verlag, Wien, 1979, Vol. 38, pp. 47–390; H. Yoshioka, T. J. Mabry, B. N. Timmermann, 'Sesquiterpene Lactones', University of Tokyo Press, Tokyo, 1973.

[18] M. Suchy, V. Herout, F. Sorm, *Collect. Czech. Chem. Commun.* **1960,** *25,* 507.

[19] K. L. Stevens, *Phytochemistry* **1982,** *21,* 1093.

[20] S. M. Kupchan, T. J. Giacobbe, J. S. Krull, A. M. Thomas, M. A. Eakin, D. C. Fessler, *J. Org. Chem.* **1970,** *35,* 3539.

[21] S. M. Kupchan, *Cancer Treat. Rep.* **1976,** *60,* 1115.

[22] A. G. Gonzalez, V. Darias, G. Alonso, G. M. Boada, M. Ferra, *Planta Med.* **1978,** *33,* 356.

[23] P. Godin, *Nature (London)* **1954,** *174,* 134.

[24] M. Kinns, J. K. M. Sanders, *J. Magn. Reson.* **1984,** *56,* 518.