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STERONES AND INDOLE ALKALOID FROM *Ailanthus altissima* CALLUS CULTURES

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Ailanthus altissima (Mill.) Swingle, Simaroubaceae, is a large tree («Tree of Heaven») distributed from Southeast Asia to China and Taiwan, but is also grown in cities in Europe and North America. It is employed as an antibacterial, anthelmintic, amoebicide, and insecticide [1–3]. Its biological activities depend mainly on quassinoids as well as on indole alkaloids, either the simple β-carbolines or the canthin-6-ones [4, 5]. Quassinoids from *A. altissima* have been shown to possess antiplasmodial activity [6, 7]. Canthin-6-one alkaloids from *A. altissima* have proven cytotoxic activity as well as anti-proliferative and antiprotozoal effects [5, 8, 9]. Cell cultures of *A. altissima* have been of particular interest for their remarkable ability to produce high yields of the canthin-6-one alkaloids normally found as very minor constituents [5]. The purpose of this study was to look at the secondary metabolites produced by callus cultures of *A. altissima* in more detail.

In the present investigation, freeze dried callus cultures of *A. altissima* were extracted subsequently with petroleum ether and chloroform. From the crude chloroform by CC on silica gel and using reverse phase HPLC, 6-β-OH-stigmasta-4-en-3-one (**1**) [10] and 6-β-OH-stigmasta-4,22-dien-3-one (**2**) [11] were obtained as amorphous white powders.

Petroleum ether and $CHCl_3$ extracts were also checked for alkaloids. TLC control of the petroleum ether fraction showed only minor amounts of alkaloids. However, from the chloroform extract several indole alkaloids could be isolated. Besides canthin-6-one, 1-methoxycanthin-6-one, 1-hydroxycanthin-6-one, canthin-6-one-3-N-oxide, and 4-methoxy-β-carboline-1-carboxylic acid methyl ester, already previously reported as constituents in callus and cell suspension cultures of *A. altissima* [5, 7], 4,8-dimethoxy-β-carboline-1-carboxylic acid methyl ester (**3**) was found. For ^{1}H NMR see [20]; ^{13}C NMR data of **3** are presented here for the first time.

So far, no reports could be found on the occurrence of **1** and **2** in cell cultures. 6-β-OH-Stigmasta-4-en-3-one (**1**) was previously detected aside from other plant sources in the fruits of *A. altissima* [12], **2** in different plants like *Phaseolus vulgaris* roots [13] and stems of *Annona cherimola* [14]. Both compounds were obtained by microbiological transformation of β-sitosterine and stigmasterine, respectively [15, 16]. Stigmast-4-en-3-one (β-sitosterone) was isolated from the heartwood of *Quassia amara*, also belonging to the Simaroubaceae family [17]. Compound **2** and the related diketone stigmasta-4,22-dien-3,6-dione are regarded as potent allelochemicals [18, 19]. So far only the β-carboline **3** was detected in the leaves of *A. altissima* [20].

Due to our investigations the production of a range of β-carboline and canthin-6-one alkaloids in callus cultures of *A. altissima* could be confirmed. The production of sterones in plant cell cultures has not been studied well before and in further investigations on their biosynthesis and biological functions plant tissue cultures of *A. altissima* might be of importance.

Plant Material. *A. altissima* seeds were obtained from mature trees from Brunswick Square, London. Authentication was achieved using plant material from Royal Botanic Gardens, Kew, Richmond. The seeds were surface sterilized in sodium hypochlorite (2 %) containing Triton X-100 for 12 min and germinated on damp Whatman No.1 filter paper in petri dishes. Aseptic cotyledon seedlings were transferred to solid (2 %) agar Murashige and Skoog's medium (Imperial Labs, U.K.) containing 1 mg×L^{-1} 2,4-dichlorophenoxyacetic acid (Flow laboratories, U.K.), 0.2 mg×L^{-1} kinetin (Flow Laboratories, U.K.), and 5% sucrose (BDH). The callus was maintained at 25°C under continuous illumination and transferred onto fresh medium every 4 weeks.

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Extraction and Isolation. Freeze dried callus cultures (624 g) of *A. altissima* were subsequently macerated with petroleum (2 ×3 L) and chloroform (5 ×2.5 L). After evaporation of the solvent the crude chloroform extract (11.86 g) was separated by CC on silica gel using a chloroform – MeOH gradient into fractions A–D. Compounds **1** (15 mg) and **2** (10 mg) were isolated from fraction B (0.7 g, chloroform – MeOH 95:5, v/v) by flash chromatography on silica gel (Sorbsil C-60, Crossfield Chemicals, U.K.) using a hexane – ethyl acetate gradient followed by RP-HPLC (Ultraspere ODS, 5 μm, 250 ×10 mm, mobile phase MeOH, 2.0 mL×min^{-1}, Rt: **1** 25.12 min, **2** 22.87 min). Compound **3** (4 mg) was obtained from fraction A (0.8 g, chloroform) containing both β-carbolines and canthin-6-one as well, by flash chromatography on silica gel (EtOAc) followed by reverse phase HPLC (Ultraspere ODS, 5 μm, 250 ×10 mm, MeOH – water 9:1, v/v, 2.2 mL/min, Rt: **3** 11.41 min.) and normal phase HPLC (LiChrospher Si 60, 5 μm, 250 × 4 mm, toluene – EtOAc 7:3 v/v, 0.9 mL/min, Rt: **3** 12.79 min). Fraction C (3.0 g, chloroform – MeOH 90:10, v/v) yielded 1-methoxycanthin-6-one as well as canthin-6-one-3-N-oxide; fraction D (1.5 g, chloroform - MeOH 90:10, v/v) yielded 1-hydroxycanthin-6-one. Their structures were established by comparison (TLC, HPLC-photodiode array detection) with authentic samples [5]; in the case of canthin-6-one-3-N-oxide, additionally by ^{1}H NMR and EI-MS [21].

6-β-OH-Stigmasta-4-en-3-one (1): DCI-MS (ammonia) *m/z* (% rel. int.) 446 (100) $[M+NH_4]^+$, 413 (13), 385 (3), 315 (3), 287 (4), 108 (3).

6-β-OH-Stigmasta-4,22-dien-3-one (2): DCI-MS (ammonia) *m/z* (% rel. int.) 444 (100) $[M+NH_4]^+$, 411 (40), 383 (10), 315 (5), 287 (7), 108 (5).

4,8-Dimethoxy-β-carboline-1-carboxylic acid methyl ester (3): DCI-MS (ammonia) *m/z* (%rel. int.) 287 (100) $[M+1]^+$, 229 (57), 199 (22), 169 (15). ^{13}C NMR ($CDCl_3$, $TMS_{int.}$, δ, ppm): 52.53 ($COO\underline{C}H_3$-1), 55.62 ($O\underline{C}H_3$-8), 56.46 ($O\underline{C}H_3$-4), 107.95 (C-7), 116.15 (C-6), 119.15 (C-11), 121.35 (C-12, -5), 122.35 (C-3), 124.08 (C-10), 130.19 (C-13), 138.09 (C-1), 146.00 (C-8), 154.49 (C-4), 166.67 ($\underline{C}OOCH_3$).

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