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BIOSYNTHESIS OF STIGMASTA-7,*E*-24(28)-DIEN-3β-OL AND 24α-ALKYL STEROLS IN *BRYONIA DIOICA*

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Key Word Index—*Bryonia dioica*; Cucurbitaceae; 24α- and 24β-alkyl sterols; phytosterol side-chain biosynthesis; stigmasta-7,*E*-24(28)-dien-3β-ol.

INTRODUCTION

We have recently reported the isolation and the structure elucidation of stigmasta-7,*E*-24(28)-dien-3β-ol (**1**) [1], which had been previously synthesized [2] and detected in small amounts in the starfish *Asterias rubens* [3], but never found in the plant kingdom. 24-Ethylidene sterols, with the *Z*-configuration, have been obtained from several sources [4] and they were considered the precursors of both 24α-alkyl sterols in higher plants and poriferasterol (24β-alkyl sterol) in *Ochromonas malhamensis* [5]. In the latter organism incorporation studies indicated that 28-isofucosterol (with the *Z*-configuration) appeared to be more readily utilized than fucosterol (with the *E*-configuration) [6].

The co-occurrence of stigmasta-7,*E*-24(28)-dien-3β-ol (**1**) together with stigmast-7-en-3β-ol (**2**) and α-spinasterol (**3**) in *Bryonia dioica* roots [1] could suggest a precursor–product relationship as indicated for 28-isofucosterol and sitosterol in other higher plants [5]. Therefore we considered it would be of biogenetic interest to (a) compare the sterol fraction of *Bryonia dioica* at different development stages (seeds, seedlings and mature plants) and (b) feed acetate-[2-^{14}C] to both the seedlings and apical shoots of the adult plant.

RESULTS AND DISCUSSION

The seeds, seedlings, aerial tips and roots of the adult plant were separately investigated giving the following sterols characterized by their physical and spectroscopic data: stigmasta-7,*E*-24(28)-dien-3β-ol (**1**), stigmast-7-en-3β-ol (**2**), α-spinasterol (**3**), (24ξ)-24-methyl-5α-cholest-7-en-3β-ol (**4**), Δ^7-avenasterol (**5**), (24*S*)-24-ethyl-5α-cholest-7,25-dien-3β-ol (**6**) and (24*S*)-24-ethyl-5α-cholest-7,22,25-trien-3β-ol (**7**). GLC, MS, IR and ^{1}H NMR (250 MHz) of the above compounds were in accord with those published [1, 7–13]. The stereochemistry at C-24 was assigned on the basis of the high field ^{1}H NMR spectra as recently reported [8, 9, 14]. The relative percentage of each sterol at different developmental stages of the plant is shown in Table 1.

These results proved that *Bryonia dioica*, like other Cucurbitaceae [15], contains Δ^7-sterols, whereas Δ^5-sterols which are more common in higher plants, were not detected. In addition a peculiarity of the *Bryonia dioica* seeds and seedlings is the presence of both 24α- and 24β-alkyl sterols, which usually occur in higher plants or in algae, respectively [5]. However, in the mature tissue of the plants (roots and apical shoots) only the 24α-alkyl sterols are present as in the case of *Cucurbita pepo* [14]. This fact was called 'evolutionary recapitulation' since sterols of the majority of the investigated non-vascular plants contain only 24β-alkyl sterols [14]. The roots and apical shoots of the adult plant are also characterized by the presence of the 'unnatural' sterol stigmasta-7,*E*-24(28)-dien-3β-ol (**1**) which accompanies the more common sterols stigmast-7-en-3β-ol (**2**) and α-spinasterol (**3**) [1].

The distribution of the radioactivity among the vari-

Nomenclature. α-Spinasterol: (24*S*)-24-ethyl-5α-cholesta-7,*E*-22-dien-3β-ol; Δ^7-avenasterol: stigmasta-7,*Z*-24(28)-dien-3β-ol.

Table 1. Sterol composition and incorporation of acetate-[2-^{14}C] into sterols of *Bryonia dioica*

	Relative quantity (%)				Acetate-[2-^{14}C] incorporation Radioactivity distribution (%)		
Compounds	Seeds	Seedlings	Roots*	Apical shoots	Seedlings A†	Seedlings B‡	Apical shoots*‡
4	2.7	—	—	—	—	—	—
2	5.4	14.8	44	35.1	0.0	0.0	36
3	25.1	39.8	34	26.6	26.1	59.7	5
6	7.35	9.7	—	—	20	6	0.0
5	1.83	1	—	—	3.4	0.0	0.0
1	9.42	tr	22	36.5	0.0	0.0	59
7	48	36.5	—	1	50.5	34.3	—

* Roots and apical shoots were collected from an old plant.

† Incubation time 36 hr.

‡ Incubation time 4 days.

ous sterols following administration of acetate-[2-^{14}C] to the seedlings and apical shoots of *Bryonia dioica* at different incubation times (36 hr or 4 days) is shown in Table 1. The radiochemical purity of each compound was established by crystallization of the appropriate steryl acetate sample with carrier compound to constant specific radioactivity. Further proof of the labelling of α-spinasterol (**3**) was obtained by ozonization of **3** in the presence of carrier material followed by Zn reduction of the labelled ozonide. Labelled **5** and **6** were allowed to react with OsO_4 and the crude reaction mixture treated with $NaIO_4$ and reacetylated giving the 24-oxo-cholest-7-en-3β-yl acetate and 26-nor-25-oxo-24-ethyl-cholest-7-en-3β-yl acetate which were both labelled.

Our data indicated that the sterol fraction of the 2-day-old seedlings consisted primarily of labelled **3** and **7**, whereas (24*S*)-24-ethyl-5α-cholest-7,25-dien-3β-ol (**6**) and Δ^7-avenasterol (**5**), supposed precursor of **3** and **7**, disappeared after a prolonged incubation time (4 days).

It is noteworthy that stigmast-7-en-3β-ol (**2**) and stigmasta-7,*E*-24(28)-dien-3β-ol (**1**), which were not radioactive at the seedling state, were actively synthesized by the adult tissue of the plant. By contrast α-spinasterol (**3**) and the 24β-ethyl sterols (**6**, **7**) were almost exclusively formed during seed germination. Consequently two distinct biosynthetic pathways, **5**→**3** and **1**→**2** might be operative in the course of the vegetative life of *Bryonia dioica*.

EXPERIMENTAL

General. Methods (^{1}H NMR, MS, IR, GLC, GC–MS, TLC and PLC) were generally as previously described [1]. Radioactivity on the plates was detected by TLC scanner and assayed by liquid scintillation counting using Permafluor Packard as liquid scintillator. MeCOONa-[2-^{14}C] (sp. act. 50 mCi/mM) was purchased from Sorin (Saluggia). The following TLC solvent systems were used: (*a*) CH_2Cl_2; (*b*) $CHCl_3$ free from EtOH; (*c*) cyclohexane–C_6H_6 (7:3) on continuous TLC for 14 hr; (*d*) cyclohexane–EtOAc (85:15). *Bryonia dioica* seeds were obtained from the Botanisher Garten der Universität, Karlsruhe (W. Germany). The seeds were soaked for 24 hr in H_2O, planted in moist vermiculite and germinated at 27° under light. Apical shoots were excised from a 5-year-old plant, 2 months after sprouting.

Extraction and fractionation. The 2-day-old seedlings as well as the seeds and shoots were homogenized and extracted with boiling 80% aq. EtOH, saponified and the neutral lipids extracted with petrol. PLC on Si gel (system *d*) gave the 4-desmethyl sterol fraction which was acetylated (Py–Ac_2O). Steryl acetates were separated by multiple argentation TLC (systems *b* and *c*) into 7 compounds (**1**–**7**) which were identified by GLC, MS, ^{1}H NMR and IR spectroscopy.

Tracer experiments. MeCOONa-[2-^{14}C] (1×10^7 dpm) soln was fed to 25 2-day-old seedlings under continuous light for 36 hr or 4 days. Ten cut ends of excised shoots were placed in a flask containing 30 ml MeCOONa-[2-^{14}C] (9×10^7 dpm) soln and were allowed to absorb the soln at 26° under light for 4 days. After incubation the seedlings or the shoots were extracted and fractionated as described above, giving the labelled steryl acetates (Table 1) which, after crystallization with the corresponding carriers, led to a constant sp. act. The radioactive **5**- and **6**-acetates (1×10^5 dpm) obtained from 2-day-old seedlings were diluted with carrier compounds and allowed to react with OsO_4 in Py as previously described [1]. The crude reaction mixture, treated with $NaIO_4$ and reacetylated, gave 24-oxo-cholest-7-en-3β-yl acetate (6×10^3 dpm) and 26-nor-25-oxo-24β-ethylcholest-7-en-3β-yl acetate (3.53×10^4 dpm) which were characterized by IR, ^{1}H NMR and MS [1, 7]. Using the same conditions, stigmasta-7,*E*-24(28)-dien-3β-yl acetate (1.53×10^5 dpm), obtained from the apical shoots, was transformed into 24-oxo-cholest-7-en-3β-yl acetate (5×10^4 dpm). Radioactive α-spinasteryl acetate (1×10^5 dpm) was diluted with carrier **2**, dissolved in dry CH_2Cl_2 containing Py and ozonized at −80° (dry ice–Me_2CO). Zn dust reduction of the ozonide yielded the labelled 5α-bisnorchol-7-en-22-al-3β-yl acetate (2.5×10^4 dpm) which was identified by IR, ^{1}H NMR and MS [16].

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