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ERGOSTA-5,23-DIEN-3β-OL AND ERGOSTA-7,23-DIEN-3β-OL, TWO NEW STEROLS FROM *ZEA MAYS* ETIOLATED COLEOPTILES

FRANÇOISE SCHEID and PIERRE BENVENISTE

Institut de Botanique, Laboratoire de Biochimie Végétale, E.R.A. du CNRS No. 487, 28, rue Goethe, 67083-Strasbourg, France

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Key Word Index—*Zea mays*; Gramineae; phytosterol biosynthesis; 24-methyl-Δ^{23}-sterols; ergosta-5,23-dien-3β-ol; ergosta-7,23-dien-3β-ol.

Abstract—Ergosta-5,23-dien-3β-ol and ergosta-7,23-dien-3β-ol were identified for the first time in maize etiolated coleoptiles. They represent more than 11% of the total 4-desmethyl sterol fraction. It is suggested that they could play some role in the biosynthesis of 24-methyl sterols of this material.

INTRODUCTION

Etiolated maize coleoptiles represent an actively growing organ. In order to study relationships existing between cell elongation and sterol synthesis, we proceeded first to a careful analysis of the sterols contained in this material. In addition to the usually occurring sterols found equally in leaves and shoots [1–3], male and female inflorescences [4], pollen [4] and tassels [5], we detected two new sterols, **1** and **2**, which constituted 9 and 2% respectively of the total amount of free sterols. Traces of **1** had been reported previously in maize pollen [4] but complete identification was not performed.

RESULTS

The 4-desmethyl sterols were extracted and separated by conventional methods. The 4-desmethyl steryl acetates separated into four major bands by $AgNO_3$–Si gel TLC. Band 1 (R_f 0.72) contained sitosteryl, campesteryl and stigmasteryl acetates (83% of total steryl acetates), band 2 (R_f 0.44) contained the two unknown products (11%), band 3 (R_f 0.38) contained isofucosteryl acetate (2%) and finally band 4 (R_f 0.18) contained 24-methylene-cholesteryl, episteryl and other minor unknown steryl acetates, (2%). A second $AgNO_3$–Si gel TLC (solvent $CHCl_3$) allowed us to separate the two new steryl acetates, **1a** (mp 122–124°, $[\alpha]_D$ −41°) and **2a**. The MS of **1a** showed that it possessed a C_{28} skeleton with two double bonds (*m/e* 380, 90%, M − HOAc), one of which was located in the side chain (peak at *m/e* 255, 9%, M − HOAc − C_9H_{19}) [6]. A prominent fragment ion at *m/e* 283 (90%, M − HOAc − C_7H_{13}) suggested that the side chain double bond was located at C-23 in order to facilitate cleavage at the C-20, C-22 bond. The other double bond was most probably located at C-5 since no molecular peak could be detected at *m/e* 440. The MS of **2a** showed evidence for a C_{28} skeleton with a double bond in the side chain, probably located at C-23 (*m/e* 283, 60%, M − HOAc − C_7H_{13}) and the other probably situated at C-7 for the following reasons. Firstly a molecular peak was observed at *m/e* 440 (15%) and secondly a peak at *m/e* 313 (100%, M − side chain − 2H), characteristic of a Δ^7 double bond in addition to a double bond in the side chain [6] was observed.

Chemical shifts of the proton signals of **1a** and **2a** in the ^1H NMR spectra are given in Table 1. The spectrum of **1a** showed a three proton resonance at δ 0.683 and a three proton resonance at 1.017 corresponding to methyls C-18 and C-19 in a Δ^5 double bond containing sterol [7]. Further resonances at δ 2.303 (C-4βH), 2.332 (C-4αH), and 5.384 (C-5H) are consistent with Δ^5 unsaturation [8]. Other resonances were observed at δ 1.544 (one vinylic methyl), 5.135 (one vinylic proton in the side chain) and 2.232 (C-25H). This latter proton gave a well resolved septet resulting from coupling of the C-25H with the six equivalent protons of C-26 and C-27. This was demonstrated using the spin decoupling procedure; after irradiation of the region of the C-26 and C-27 methyls, the septet corresponding to C-25H disappeared and was replaced by a single signal; when the area of the C-25H was irradiated, this lead to disappearance of the doublet resulting from coupling of the C-26 and C-27 methyls with the C-25H and to the appearance of a single signal. This allowed the location of the double bond of the side chain to be proposed as position C-23. On the basis of the above data, the steryl acetate was tentatively assigned the structure **1a**. The ^1H NMR spectrum of **2a** (Table 1) showed a three proton resonance at δ 0.538 and a three proton resonance at 0.808 corresponding to methyls C-18 and C-19 in a Δ^7 sterol [7]. The other resonances were similar or identical to those of **1a** except that the vinyl proton at C-7 of **2a** resonated at higher field than the vinyl proton at C-6 of **1a**. On the basis of the data presented in Table 1, the steryl acetate was assigned the structure **2a**. The configuration of the Δ^{23} double bond was not determined in this study.

Synthetic ergosta-5,23-dien-3β-yl acetate was prepared following isomerization of ergosta-5,24(28)-dien-3β-yl acetate (**3a**) with iodine in benzene under reflux [9]. This reaction yielded a mixture of dienes differing in the position of the side chain double bond. Separation of this mixture by $AgNO_3$–Si gel TLC gave a product (R_f 0.44),

Table 1. ^{1}H NMR chemical shifts of the proton signals of **1a**, **2a** and **4a**

	C-18	C-19	C-21	C-26 and C-27	C-28	C-25	C-4α	C-4β	C-3α	C-23	C-6
Ergosta-5,23-dien-3β-yl-acetate (**1a**)	0.683 *s*	1.017 *s*	0.887 (*d*, *J* = 6.5*)	0.985 (*d*, *J* = 6.8)	1.544 *s*	2.232 (*septet*, *J* = 6.7)	2.332 *s*	2.303 *s*	4.623 *m*	5.135 (*t*, *J* = 6.5)	5.384 (*d*, *J* = 5)
Ergosta-7,23-dien-3β-yl-acetate (**2a**)	0.538 *s*	0.818 *s*	0.891 (*d*, *J* = 6.5)	0.985 (*d*, *J* = 6.8)	1.547 *s*	2.234 (*septet*, *J* = 7)			4.695 *m*	5.135 (*t*, *J* = 6.5)	5.144 *d*
Ergosta-5,24(25)-dien-3β-yl-acetate (**4a**)	0.679 *s*	1.019 *s*	0.960 (*d*, *J* = 7)	1.629 *s*	1.629 *s*		2.332 *s*	2.303 *s*	4.624 *m*		5.384 (*d*, *J* = 5)

Spectra were determined at 250 MHz in $CDCl_3$. Chemical shifts are given in δ (ppm).
* Coupling constants in Hz.

mp 122–124°, $[\alpha]_D$ −41°, (15% yield) and another product (R_f 0.53), mp 140–141°, (15% yield). The more polar product had identical retention time (RR_t OV17 = 1.74), MS, ^{1}H NMR and IR spectra as the natural **1a**. Thus it could be concluded that this product was ergosta-5,23-dien-3β-yl acetate and that the configuration of the Δ^{23} double bond was the same in the synthetic steryl acetate as in **1a**. The ^{1}H NMR spectrum of the less polar product was identical to that of ergosta-5,24(25)-dien-3β-yl acetate (**4a**), isolated previously from *Withania somnifera* and which has been proposed as a possible intermediate in the biosynthesis of 24-methyl sterols in higher plants [8, 10]. As ergosta-8,23-dien-3β-ol (ascosterol), a sterol structurally related to **1** had been previously reported in yeast [11], a fungal origin for **1** was considered. Maize seeds were first treated for 1 hr with $Ca(ClO_2)$ and then allowed to germinate in aseptic conditions. Coleoptiles were treated as previously described. As the same concentrations of **1** and **2** were found in the sterile as in the non-sterile coleoptiles, it was concluded that **1** and **2** were biosynthesized by maize coleoptiles themselves.

DISCUSSION

The presence of **1** and **2** in maize coleoptiles raises the problem of their biosynthesis and of their involvement in the biosynthesis of 24-methyl sterols of this material. Two pathways can be proposed to explain the biosynthesis of **1** or **2** (Scheme 1). Route *a* implies that Δ^{23}-sterols are products of the C-24 methylation reaction whereas route *b* involves the formation of a 24-methylene sterol followed by an isomerization reaction leading to Δ^{23}-sterols. 24-Methyl sterols have been shown to be a mixture of epimers at C-24 [12]. Some evidence has been given [8, 10, 13] that the major pathway leading to 24α-methyl sterols probably involves the intermediacy of a $\Delta^{24(25)}$-sterol. It has been proposed also that 24β-methyl sterols arise through $\Delta^{25(27)}$-sterols [13]. Another possibility could be that 24β-methyl sterols arise through Δ^{23}-sterols as suggested by the simultaneous presence of Δ^{23}-sterols and of 24-methyl sterols in etiolated maize coleoptiles. It may be recalled that in this context the intermediacy of Δ^{23}-sterols in the biosynthesis of 24-alkyl sterols has been proposed previously [14].

EXPERIMENTAL

Plant material. Maize seeds (*Zea mays* cv INRA 258) were allowed to germinate in the dark at 25°. The coleoptiles were excised after 6 days of germination.

Isolation and identification of sterols. Most of the techniques used in the present work have been described previously [15]. **Ergosta-5,23-dien-3β-yl-acetate (1a)**: MS *m/e* (rel. int.): 380 (M^+ −60) (90), 355 (9), 296 (12), 283 (90), 259 (6), 255 (9), 253 (32), 227 (7), 217 (3), 215 (9), 213 (9), 159 (27), 133 (54), 81 (100). **Ergosta-7,23-dien-3β-yl-acetate (2a)**: MS *m/e* (rel. int): 440 (M^+) (15), 425 (11), 394 (20), 380 (11), 343 (19), 313 (100), 283 (60), 255 (12), 253 (22), 227 (14), 215 (13), 213 (21), 201 (18), 159 (24), 133 (22), 81 (9). **Ergosta-5,24-dien-3β-yl-acetate (4a)**: MS *m/e* (rel. int.): 380 (M^+ −60) (72), 365 (18), 296 (100), 281 (30), 259 (18), 255 (16), 253 (36), 229 (20), 213 (32).

NOMENCLATURE

Sitosterol = (24*R*)-24-ethyl-5α-cholest-5-en-3β-ol; campesterol = (24*R*)-24-methyl-5α-cholest-5-en-3β-ol; 22-dihydrobrassicasterol = (24*S*)-24-methyl-5α-cholest-5-en-3β-ol; isofucosterol = stigmasta-5,*Z*-24(28)-dien-3β-ol; episterol = ergosta-7,24(28)-dien-3β-ol; 24-methylene cholesterol = ergosta-5,24-(28)-3β-ol.

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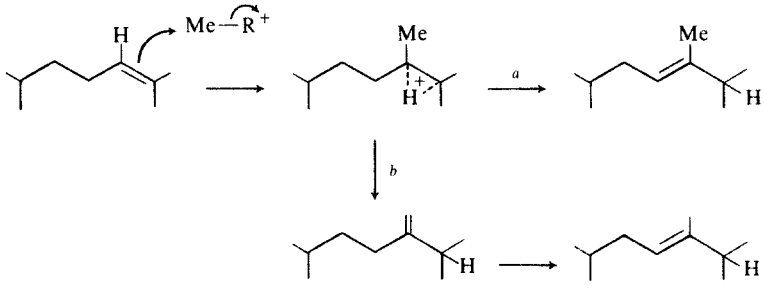
REFERENCES

1. Rohmer, M., Ourisson, G. and Brandt, R. D. (1972) *Eur. J. Biochem.* **31**, 172.



Scheme 1. Possible routes for the biosynthesis of the 24-methyl-Δ^{23}-sterols in *Zea mays*.

2. Kemp, R. J. and Mercer, E. I. (1968) *Biochem. J.* **110**, 111.
3. Kemp, R. J., Goad, L. J. and Mercer, E. I. (1967) *Phytochemistry* **6**, 1609.
4. Knights, B. A. and Smith, A. R. (1976) *Planta* **133**, 89.
5. Comita, J. J. and Klosterman, M. J. (1976) *Phytochemistry* **15**, 917.
6. Wyllie, S. G. and Djerassi, C. (1968) *J. Org. Chem.* **33**, 305.
7. Rubinstein, I., Goad, L. J., Clague, D. H. and Mulheirn, L. J. (1976) *Phytochemistry* **15**, 195.
8. Lockley, W. J. S., Roberts, D. P., Rees, H. H. and Goodwin, T. W. (1974) *Tetrahedron Letters* 3773.
9. Ikekawa, N., Honma, Y., Morisaki, N. and Sakai, K. (1968) *J. Org. Chem.* **33**, 305.
10. Armarego, W. L. F., Goad, L. J. and Goodwin, T. W. (1973) *Phytochemistry* **12**, 2181.
11. Fuerst, W. (1966) *Ann. Chem.* **699**, 206.
12. Nes, W. R., Krevitz, K. and Behzadan, S. (1976) *Lipids* **11**, 118.
13. McKean, M. L. and Nes, W. R. (1977) *Phytochemistry* **16**, 683.
14. Boid, R., Rees, H. M. and Goodwin, T. W. (1974) *Biochem. Soc. Trans.* **2**, 1066.
15. Schmitt, P. and Benveniste, P. (1979) *Phytochemistry* **18**, 445.