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SHORT REPORTS

BIOSATURATION OF CYCLOPROPENE FATTY ACIDS IN GERMINATING COTTONSEED

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Key Word Index—*Gossypium hirsutum*; *Sterculia foetida*; cottonseed; cyclopropene fatty acids.

Abstract—*cis*-Hydrogenation of cyclopropenoid fatty acid (CPFA) to cyclopropane fatty acid takes place by enzymic treatment indicating that the enzyme present in germinating cottonseed is stereospecific in action resulting in the *cis*-isomer only.

INTRODUCTION

The biogenesis of cyclopropene fatty acids (CPFA) via desaturation of cyclopropane fatty acids is well documented [1–4] but the mechanism of biodegradation of CPFA is not well understood. We have earlier shown that the CPFA content of the germinating cottonseed decreased significantly in a short four day-period, independent of disappearance of normal fatty acids [5, 6]. On the other hand, during the period of rapid lipid formation in maturing cottonseed, CPFA is biosynthesized at a rate similar to normal fatty acids but unlike the latter decreases after reaching a peak value before attaining a steady value in the final stages [5]. These observations indicate specific metabolic changes of CPFA. We now report *in vitro* experiments which demonstrate that the initial metabolic step consists of saturation of CPFA to *cis*-cyclopropane fatty acids by a reductase present in cottonseed. This enzyme appears to be highly substrate specific as normal unsaturated fatty acids are not saturated under identical conditions.

RESULTS AND DISCUSSION

During a four-hr treatment of cottonseed and *Sterculia foetida* seed oils with the water extract of defatted cottonseed meal, the CPFA contents of the oils were reduced by 90.2 and 91.7%, respectively. The salt and phosphate buffer extracts of the defatted seed powder behaved similarly in the reduction of CPFA content of the seed oils. The CPFA substrate was unaffected in control experiments with distilled water alone.

The ^{1}H NMR spectrum of the methyl sterculate-CPFA moiety showed the characteristic [7–9] low field signal at δ0.8. However, the product of its treatment with the seed extract showed, in addition to a significantly reduced signal at δ0.8, two other prominent signals at 0.6 and −0.33. The highly shielded signal at δ0–0.33 is unique in organic chemical compounds and is characteristic of a *cis*-cyclopropane moiety [9]. The signal is attributed to one of the hydrogens *cis* to the dual hydrophobe groups of the *cis*-cyclopropane system; this signal is absent in a *trans*-substituted cyclopropane system. Thus, the ^{1}H NMR analysis unequivocally confirmed the presence of a reductase enzyme in germinating cottonseed. The reduced response of the product to the Halphen test [10], as well as the NMR data, showed that CPFA was gradually hydrogenated stereospecifically to a *cis*-cyclopropane moiety.

In vitro tests conducted under identical conditions using linseed oil or its methyl esters as substrate did not reveal any change in fatty acid composition indicating the substrate specificity of the enzyme.

A low M_r for the enzyme was indicated by the failure to precipitate it from water extracts by ammonium sulphate. Some degree of purification was achieved by dialysing a water-extract of seed against distilled water over night and the water solubles were again dialysed against 0.025 M phosphate buffer. The concentrate was then fractionated on a DEAE Cellulose (HB-70 grade) column (2.5 × 30 cm) which was previously equilibrated with 0.025 M phosphate buffer. The fractions having 3.6–5.5 mg of protein/ml obtained during elution with 0.055 M phosphate buffer had the highest reductase activity amongst the different fractions.

The present observation of enzymic reduction of CPFA to *cis*-cyclopropane derivatives explains our earlier observation of the rapid disappearance of CPFA in germinating cottonseed [6]. This bioreduction of CPFA is a reversal of desaturation which is the final step in CPFA biosynthesis [1]. While dehydrogenases are known to occur in nature, the occurrence of reductases in seeds has not been reported. Modification of the enzyme can conceivably change the rigid substrate requirement to cover other types of unsaturated lipids for biotechnological modifications of fats.

EXPERIMENTAL

Sterculia foetida seed oil (63% CPFA) or methyl sterculate (96% pure) prepared [11] from the oil or cottonseed oil (0.3%

CPFA), respectively (were used as substrate. The CPFA content of the substrate and product was determined by the AOAC procedure [10]. The crude enzyme fraction was obtained by aqueous extraction of the dehulled air-dried and defatted (solvent system $CHCl_3$–MeOH, 2:1) germinating cottonseed. For this purpose, well developed delinted cottonseed of Hybrid-4 variety (*Gossypium hirsutum*) were allowed to germinate by keeping for 2 days between folds of wet filter paper, and the non-shell portion was air-dried and ground before defatting.

In a typical experiment, the defatted powder (10 g) was extracted with H_2O (20 ml) by vigorous agitation for 1 hr at room temp. The contents were centrifuged at $12000 \times g$ for 15 min. The supernatant (10 ml) and substrate (100 mg) were mixed and agitated in a shaker for a predetermined time at room temp. and the oily layer was extracted with hexane, washed with H_2O, dried over Na_2SO_4, filtered and the solvent removed. The residual oil was tested for CPFA content by the standard AOAC procedure [10], and also analysed by ^1H NMR. The recovery of the fatty portion after the enzyme treatment was over 90%.

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PALMITIC ACID ESTER OF SITOSTERYL 3β-GLUCOSIDE FROM *CENTAUREA REGIA*

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Key Word Index—*Centaurea regia*; Compositae; sitosteryl 3β-glucoside 6′-*O*-palmitate; steroids; triterpenes; flavones; phenolic compounds; lignan.

Abstract—A new steroid derivative, sitosteryl 3β-glucoside 6′-*O*-palmitate, together with sitosterol, sitosteryl 3β-glucoside, lupeol, taraxasterol, crysoeriol, vitexin, luteolin 3′-glucoside, *p*-hydroxybenzoic acid, vanillic acid, *p*-hydroxyacetophenone and a lignan, (−)-arctigenin, were isolated from the aerial parts of *Centaurea regia*.

INTRODUCTION

In our previous studies with *Centaurea* species we have obtained flavonoids from *C. urvillei* [1] and *C. inermis*, *C. kilea*, *C. virgata* [2] and guaianolides from *C. behen* [3], *C. kotschyii* [4]. In this first chemical investigation of *Centaurea regia* Boiss. in addition to a new steroid derivative, sitosteryl 3β-glucoside 6′-*O*-palmitate, we have obtained known flavonoids crysoeriol, vitexin and luteolin 3′-glucoside [5] and terpenic compounds lupeol, taraxasterol, sitosterol, sitosteryl 3β-glucoside, as well as phenolic compounds *p*-hydroxybenzoic acid, vanillic acid and *p*-hydroxyacetophenone and a lignan (−)-arctigenin [6, 7].

RESULTS AND DISCUSSION

The residue from ether–petrol extracts of the plant material was fractioned on a silica gel column. The compounds obtained from the column were further separated by preparative TLC. The structures of the known compounds were established by spectral methods and, except for (−)-arctigenin, by direct comparison with authentic samples. Identification of (−)-arctigenin was