amino acids and amines was examined. Table 3 shows the estimated incorporations of ^3H from spermine into 1,3-diaminopropane and β-alanine. Considerable radioactivity was incorporated into the fraction of 1,3-diaminopropane plus 1-(3-aminopropyl)-pyrroline and β-alanine. The sp. act. of β-alanine found after 24 hr incubation from 0.5 μCi/μmol spermine was 0.18 μCi/μmol (Table 3). Theoretically this value should be 0.18 μCi/μmol. However, 2–4% of the radioactivity derived from spermine-[^3H] was recovered in an unidentified compound (Table 3). The biosynthetic route of β-alanine in higher plants as suggested here seems attractive, since spermine and spermidine are widespread in various Gramineae [5]. The formation of 1,3-diaminopropane from both polyamines has been established convincingly at the enzyme level [1]. Our results leave open the question of the mechanism by which the 1,3-diaminopropane is oxidised.

EXPERIMENTAL

Plant material. Seeds of maize (*Zea mays.* L. cv Goldencross Bantam T 51) were germinated in moist vermiculite at 25° for 3 days in the dark. The seedlings were then transferred to plastic trays containing Hoagland's soln. They were grown hydroponically under continuous light (*ca* 2 klx at plant level) at 25° for 11 days. Etiolated seedlings were raised in moist vermiculite at 25° for 7 days in the dark.

Feeding experiment. 4 to 5 shoot tips, comprising the 3 developed leaves, were used. Cut ends (2 cm above the seed) of excised shoot tips were placed in a flask containing 30 ml of 1/2 strength Hoagland's soln with or without 35 mM spermine, spermidine or 1,3-diaminopropane and were allowed to absorb the soln at 25° in the dark or under light (*ca* 2 klx). The solns in the flasks were renewed every day.

Extraction and fractionation. The amino acids and amines from green shoots were determined in the EtOH-soluble fraction obtained by extracting with 99% EtOH (8 ml per g fr. wt). After centrifugation (10 min at 1000 *g*), the chlorophyll and other pigments were removed from the supernatant by extraction with toluene [6]. The amino acids and amines from the etiolated shoots were obtained according to the method ref. [7]. The extracts obtained were treated with Amberite IR-120 (H^+) [7]. Partially purified fractions of amino acids and amines were trimethylsilyated (TMSi) [8].

GLC (FID) was carried out with 3 mm id × 2 m glass columns packed with Chromosorb W coated with 10% OV-17 or SE-30.

Tracer experiments. 11-days-old shoots absorbed 35 mM radioactive spermine [3-aminopropyl-3-[^3H(C)] : sp. act. 0.5 μCi/μmol] and 1/2 strength Hoagland's soln under continuous light (as above). After 24 hr, they were transferred to another flask containing 35 mM spermine and 1/2 strength Hoagland's soln. At the end of the experimental periods, they were separated by descending PC, with *n*-BuOH–HOAc–H_2O (4:1:2) (solvent 1); and methyl cellosolve–HOPr–H_2O (14:3:3) satd with NaCl (solvent 2) [9]. After spraying with ninhydrin, spermine, 1,3-diaminopropane, 1-(3-aminopropyl)-pyrroline, and β-alanine were determined. R_fs in solvent 1 were spermine, 0.05; 1,3-diaminopropane, 0.13; β-alanine, 0.40; and 1-(3-aminopropyl)pyrroline, 0.11: R_fs in solvent 2 were 0.09, 0.27, 0.62 and 0.45 resp. 1-(3-Aminopropyl)pyrroline was obtained in the polyamine oxidase reaction. The radioactivity of the amino acids and amines was measured directly from the papers by liquid scintillation counter at 48% efficiency, with a PPO-dimethyl-POPOP-toluene scintillator.

Polyamine oxidase. Partially purified polyamine oxidase from maize shoots was prepared using the method of ref. [10].

Acknowledgements—We are greatly indebted to Professor B. J. D. Meeuse of the University of Washington, Seattle, U.S.A. for correcting this English manuscript. This work was supported by a research grant from the Ministry of Education of Japan to YS..

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Phytochemistry, 1978, Vol 17, pp. 149–150 Pergamon Press Printed in England

A SECOISOLARICIRESINOL BRANCHED FATTY DIESTER FROM *SALVIA PLEBEIA* SEED

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(*Revised received* 6 *May* 1977)

Key Word Index—*Salvia plebeia*; Labiatae; lignan; diester; *anteiso* fatty acid; 12-methyltetradecanoic acid; secoisolariciresinol.

Abstract—The structure of a second new lignan diester from *Salvia plebeia* seed has been determined. Hydrolysis of this diester yields two compounds, 12-methyltetradecanoic acid and secoisolariciresinol.

INTRODUCTION

We recently characterized a unique lignan diester (**1**) which was isolated by crystallization from the hexane extract of *Salvia plebeia* seeds [1]. Further examination of the hexane extract of this seed revealed the presence of a

second lignan diester (**2**) which was isolated by chromatography and characterized by NMR and MS. Three additional samples of *S. plebeia* seed from our collection were examined. All contained compounds **1** and **2**.

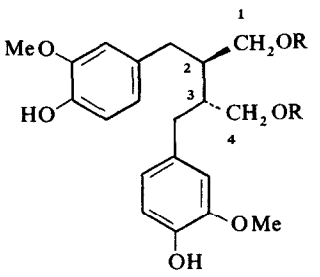
RESULTS AND DISCUSSION

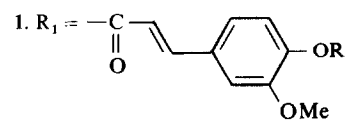
GLC of the hexane extract of *S. plebeia* seeds following saponification and esterification showed the presence of 3% methyl 12-methyltetradecanoate, an *anteiso* branched fatty ester highly unusual from a plant source [2]. PLC of the seed extract itself gave four major fractions: triglycerides; partial glycerides and free fatty acids; and lignan diesters **1** and **2**. Subsequent saponification and esterification of the triglyceride fraction revealed no branched esters.

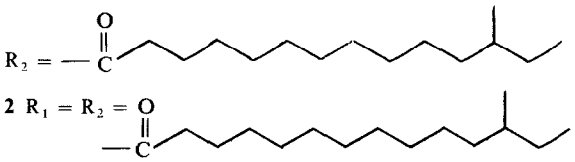
Compound **2** had an IR spectrum which indicated the presence of hydroxyl and ester carbonyl functions. Its TMS derivative eluted from 3% OV-1 columns at ~320°. The MS of **2** had a prominent apparent molecular ion (M^+) at *m/e* 810. Other intense ions included *m/e* 568 (M-242), *m/e* 326 (M-2 × 242) and a base peak at *m/e* 137. The NMR spectrum of **2** included a large singlet attributed to a long methylene chain (δ 1.24), a complex aliphatic methyl signal (δ 0.85), several aromatic protons (δ 6.8–6.4), and an aromatic methoxyl signal (3.74) and overall was very similar to the NMR of **1** except that the long chain methylene signal represented more protons and the signals assigned to the ferulic acid in **1** were absent.

Alkaline hydrolysis of **2** yielded only two products, secoisolariciresinol (**3**) and 12-methyltetradecanoic acid, which were identified by NMR, GC–MS, and GC retention time of their TMS derivatives. Compound **2** resisted all attempts to crystallize it.

Although branched chain compounds are rare in higher plants, small amounts of them have been observed in one other member of the Labiateae [3] as well as in some members [4] of the Pinaceae. The absence of branched acyl groups in the triglycerides of *Salvia plebeia* and the presence of secoisolariciresinol in members of the Pinaceae [5] suggest the possibility that branched fatty acids from plant sources, in genral, do not occur as triglycerides, but rather as esters of alcohols other than glycerol.







3. $R_1 = R_2 = H$

EXPERIMENTAL

General. All compounds were analyzed by TLC on precoated Si gel F-254 plates. NMR spectra were recorded at 100 MHz in $CDCl_3$ soln, TMS was the internal standard. MS were obtained by probe inlet or by GC MS at 70 eV. GC analyses were made on a 1.2 m × 2 mm glass column packed with 3% OV-1.

Plant material. One sample of *S. plebeia* seed was collected in India during 1974 and the other three samples from our seed collection were collected in Pakistan and Korea.

Isolation of **1** *and* **2**. Ground seed of the four samples of *S. plebeia* (10 g) was extracted for 20 hr with 250 ml hexane in a Soxhlet extractor. When the extract was cooled to room temp. and allowed to stand, **1** crystallized as a white ppt. as reported previously [1]. Chromatography of 1.0 g of the total *S. plebeia* extract on 25 g of High Folsil yielded 908 mg of triglycerides (R_f = 0.96; hexane–Et_2O, 6:4), 46 mg of partial glycerides and free acids (R_f = 0.3–0.8 three spots), 13 mg of **2** (R_f = 0.25) and 25 mg of **1** (R_f = 0.05). The other three *S. plebeia* seed oil samples gave similar TLC results. Compound **1** gave a single spot by TLC (R_f = 0.05; hexane–Et_2O, 3:2, R_f = 0.44; C_6H_6–$CHCl_3$–MeOH, 50:50:3). IR ($CHCl_3$) cm^{-1}: 3650 (OH) and 1725 (>C=O). NMR δ 7.56 (1H, *d*, *J* = 16 Hz olefinic), 7.20–6.40 (9H, *m*, aromatic), 6.24 (1H, *d*, *J* = 16 Hz olefinic), 5.97 (1H, *s*, —OH), 5.53 (2H, *s*, —OH), 4.28 (4H, *m*, —C<u>H</u>$_2$—O—), 3.89 (3H, *s*, —OC<u>H</u>$_3$), 3.74 (6H, *s*, —OC<u>H</u>$_3$), 2.67 (4H, apparent *d* Ar-C<u>H</u>$_2$—CH<), 2.25 (4H, *m*, —C<u>H</u>$_2$—CO—O and —CH<), 1.24 [*ca.* 22H, *s*, —(CH$_2$)—], 0.85 (6H, m, —C<u>H</u>$_3$ and CH—C<u>H</u>$_3$). MS *m/e* (rel. int.): 762 M^+ (7), 568 (17), 326 (25), 189 (32), 177 (55) and 137 (100). Compound **2** gave a single spot by TLC (R_f = 0.25; hexane–Et_2O, 3:2, R_f = 0.75; C_6H_6–$CHCl_3$–MeOH, 50:50:3). IR ($CHCl_3$) cm^{-1}: 3650 (OH) and 1725 (C = O). NMR δ 6.80–6.40 (6H, *m* aromatic), 4.04 (4H, *m*, C<u>H</u>$_2$—O—), 3.74 (6H, *s*, ArOC<u>H</u>$_3$), 2.58 4H, *d*, Ar—C<u>H</u>$_2$—CH<), 2.25 (4H, *m*, CH$_2$—CO—O and CH<), 1.24 [~40H, *s*, —(CH$_2$)—], 0.85 (6H, *m*, -Me and >CH—Me). MS *m/e* (rel. int.): 810 M^+ (15), 568 (16), 326 (23), 189 (41), 137 (100).

Saponification of **2**. Compound **2** (10 mg) was saponified by refluxing for 2 hr in a soln of N aq. KOH (10 ml) and MeOH (10 ml). H_2O was added and the soln was extracted with $CHCl_3$. The two products were separated by PLC and identified by GC–MS and by TLC comparison with authentic samples.

Acknowledgement—We thank Dr. D. Weisleder for NMR spectra.

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