(6.2 mg) in a mixture of EtOH (1 ml) and H_2O (1 ml) was hydrogenated at room temp. until uptake of H_2 had ceased (2 hr). After removal of the catalyst under N_2 using glass paper, evaporation at < 1 mm pressure gave the phenolic indole (**2**) as a colourless glass; IR ν_{max}^{KBr} cm^{-1}: 3410 *s*, 1722, 1630 *s*, 1555; MS *m/z* (rel. int.): [262.0953 $[M]^+$ (14), $C_{13}H_{14}N_2O_4$ requires 262.0953], 203.0581 $[M-MeCONH_2]^+$ (19), 146.0602 [*β*-(6-hydroxyindolyl)CH_2]$^+$ (100), 133.0529 $[M-CH_2=C(NHAc)CO_2H]^+$ (4.8), 117.0574 [*β*-(6-hydroxyindolyl)$CH_2-CHO]^+$ (2.6). This was identical (TLC behaviour, UV, NMR spectra) with a sample of **2** isolated as above.

N-*Acetyl*-6-*methoxy*-DL-*tryptophan methyl ester* (**3**). Methylation of the above phenolic acid (**2**, 5 mg) with ethereal CH_2N_2 gave the ether ester (DL-**3**), R_f 0.3 on TLC using Si and EtOAc (orange spot on development with Ce^{4+} changing to green on heating); IR $\nu_{max}^{CDCl_3}$ UV and NMR spectra identical to those of a sample of the ether ester prepared from the metabolite **2**. (Found: $[M]^+$ at *m/z* 290.1276. $C_{15}H_{15}N_2O_4$ requires 290.1266.)

Preparation of the monophenoloxidase. Cultures of strain AJC 7.46 (*ivo A1 orn B7 brl A42*) or, for the monophenol oxidase free control, strain G 841, were grown as in the production of *N*-acetyl-6-hydroxytryptophan (**2**) but for only 2 days. The resulting mycelial mat was ground in a mortar with sand and Tris–maleic buffer, pH 7.0 at 4°. The crude extract was centrifuged for 5 min at 2500 *g* and in some cases dialysed against the extraction buffer in the cold for 1 hr. Enzyme activity was assayed by adding enzyme preparation (0.1 ml) to a mixture of 0.5 M Tris–maleic buffer, pH 7.0 (1.9 ml), and an aq. soln (1 ml) containing 4-methoxyphenol (25 mg) and catechol (0.2 mg). The formation of the brown oxidation product was followed as *A* at 470 nm in a Unicam SP 1800 spectrophotometer.

Enzymic oxidation of **2**. The oxidation of purified natural **2**, on addition of monophenol oxidase preparations, could be followed polarographically and its destruction could be monitored by chromatography using diazotized sulphanilic acid for detection. Control enzyme preparations from *ivo B* strain, which lack monophenoloxidase were inactive and **2** could still be detected on chromatograms of the mixture.

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TOTAL SYNTHESIS OF SYLVAMIDE, A *PIPER* ALKAMIDE

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Key Word Index—*Piper sylvaticum*; Piperaceae; alkamide; sylvamide; *N*-isobutyl-4,5-dihydroxy-2(*E*)-decenamide.

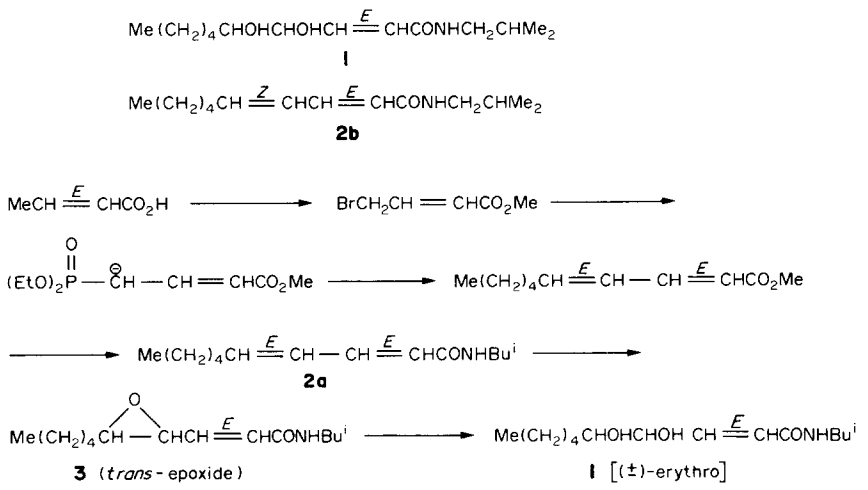
Abstract—The structure, *N*-isobutyl-4, 5-dihydroxy-2 (*E*)-decenamide, for sylvamide is confirmed by its total synthesis. The *erythro* stereochemistry is also established by comparison of the properties of the natural and synthetic samples.

INTRODUCTION

During the chemical investigation on the seeds of *Piper sylvaticum* Roxb. we isolated a new alkamide, sylvamide (**1**), whose structure was established by spectral studies and degradation experiments [1]. In the present communication we report the confirmation of its structure and the establishment of the relative stereochemistry of the two chiral centres from its total synthesis by a stereospecific pathway.

RESULTS AND DISCUSSION

The scheme formulated for the synthesis required an *N*-



Scheme 1.

isobutyldeca-2, 4-dienamide (**2a** or **2b**), as the immediate precursor. Starting from pure **2a**, in which the $\Delta^{4,5}$ bond was *E*, a (±)-*erythro*- mixture was synthesized. A stereospecific *trans* hydroxylation procedure via epoxidation was followed. Using molar proportions of the substrate and *m*-chloroperbenzoic acid under controlled condition (cf. Experimental) the 4, 5-epoxyamide, (**3**), was obtained as the only product. The $\Delta^{2,3}$ bond is less reactive due to its conjugation with the carbonyl function. The cleavage of the epoxide ring to a diol without affecting the rest of the molecule was achieved under very mild conditions. A *ca* 2% perchloric-acid solution in tetrahydrofuran containing a few drops of water was used for this purpose and the reaction was carried out at a low temperature (10–15°). Perchloric acid was chosen becasue the perchlorate ion is a poor nucleophile. The construction of the *E*-4, 5-double bond in the synthesis of **2a** was accomplished by the highly stereoselective Horner–Emmons modification [2] of the Wittig reaction. This involved the condensation of *n*-hexanal with the stabilized phosphonate anion corresponding to methyl-γ-diethylphosphonocrotonate (**4**), generated by the action of sodium hydride in DMF. The resulting product, methyl-2(*E*), 4(*E*)-decadienoate (**5**), was transformed into its isobutylamide (**2a**) by hydrolysis and conversion into the acid chloride by oxalyl chloride in benzene followed by treatment with isobutylamine in ether. The synthetic scheme is shown in Scheme 1.

The synthetic sample was identical with the natural one in TLC behaviour, IR (superimposable), ^{1}H (80 MHz) and ^{13}C(20 MHz) NMR spectral data. The identity (within ±δ0.1) of the ^{13}C chemical shifts of the synthetic and natural products proved conclusively that the compounds had the same structure and stereochemistry. However, the two samples differed in some physical constants, viz., mp and specific rotation. A mmp determined with a *ca* 1:1 composition of the naturally occurring example, mp 143–144°, $[\alpha]_D$ −2° and racemic synthetic sample, mp 122°, showed a depression to 131°. This could be rationalized by assuming that the natural compound was an optically pure *erythro* isomer and the synthetic one was a racemic mixture.

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR spectra recorded at 20 MHz and ^{13}C NMR spectra at 20 MHz in d_6-DMSO. Si gel was used for CC and Si gel G for TLC. Spots were detected by I_2 vapour. Extracts were dried over dry Na_2SO_4.

Methyl-γ-bromocrotonate. Crotonic acid (43 g, 0.5 mol) was mixed with dry MeOH (75 ml, 2 mol) and conc. H_2SO_4 (2.7 ml, 5 g). The mixture was refluxed for 12 hr. *Ca* 100 ml H_2O was then added. The ester layer was separated and the aq. layer extracted with 2 × 30 ml CH_2Cl_2. The ester and organic extracts were mixed and washed with aq. $NaHCO_3$ soln followed by H_2O. Solvent was removed and the residue on distillation gave methyl crotonate (41 g, 82%) at 118–120°.

To methyl crotonate (40 g, 0.4 mol) in dry CCl_4 (*ca* 150 ml) NBS (72 g, 0.405 mol) and azobisisobutyronitrile (*ca* 4 mg) were added. The mixture was refluxed over a 300 W tungsten filament lamp till the reaction was complete. The filtrate upon distillation under red. pres. gave methyl-γ-bromocrotonate (43 g, 63%) at 77–80°/8–10 mm. ^{1}H NMR ($CDCl_3$) δ: 3.96 (*d*, *J* = 7.2 Hz, H-4), 6.81 (*d* of *t*, *J* = 15.4, 7.2 Hz, H-3), 5.97 (*d*, *J* = 15.4 Hz, H-2), 3.70 (*s*, –COOC$\underline{H}_3$).

Methyl-γ-diethylphosphonocrotonate. Methyl-γ-bromocrotonate (18 g, 0.1 mol) was added dropwise to triethylphosphite (18.2 g, 0.104 mol) with stirring. After addition the mixture was heated at 120–130° for 2 hr. EtBr escaped. The excess triethylphosphite was removed at red. pres. (H_2O suction). The residue on distillation afforded the product at 180–182°/10–11 mm. Yield 17.5 g (75%). IR ν_{max} cm^{-1}; 1725, C=O; 1658, 968, *trans* C=C; 1250, P=O and C=O; 1025, P=O.

Methyl-2 (E), 4 (E)-decadienoate (**5**). NaH (55% in mineral oil, 2.3 g, *ca* 0.05 mol, washed with dry C_6H_6) was placed in dry DMF (*ca* 70 ml) under N_2. The slurry was stirred magnetically at 0°. Methyl-γ-diethylphosphonocrotonate (12.0 g, 0.05 mol) in DMF (*ca* 10 ml) was added dropwise. Stirring was continued for 4 hr. Freshly distilled *n*-hexanal (5 g, 0.05 mol) was added dropwise. The mixture was kept for 16 hr at room temp., then diluted with H_2O and extracted with Et_2O. The dried extract was concd and chromatographed. The desired product (3.35 g, 41%) was obtained in petrol–C_6H_6 (1:1) eluates, IR ν_{max} cm^{-1}: 1725, C=O; 1638 and 1622; C=C, 1260, C–O; 1000, *trans*-diene.

N-*Isobutyl*-2(E), 4(E)-*decadienamide* (**2a**). The ester, **5** (2.6 g, 0.015 mol) was refluxed with 8% ethanolic KOH (*ca* 50 ml) for 4 hr. The EtOH was removed under red. pres. H_2O (*ca* 40 ml) was added, the soln was neutralized with aq. HCl (1:1) and thereafter extracted with Et_2O. The organic layer was washed with H_2O, dried and concd.

The crude acid was treated with excess oxalyl chloride (2.5 ml, 0.03 mol) in dry C_6H_6 (*ca* 30 ml) at room temp. for 30 min and then refluxed for another 30 min. Removal of the solvent and excess of the reagent furnished the acid chloride as an oily residue. This was taken up in dry Et_2O (20 ml) to which was added, with stirring, isobutylamine (4 ml, 0.04 mol) in dry Et_2O (*ca* 10 ml). After 1 hr the mixture was poured into H_2O (*ca* 50 ml) and extracted with Et_2O. The extract was washed successively with 1 N H_2SO_4, aq. $NaHCO_3$ soln and H_2O, concd and then chromatographed. The product, a colourless solid, mp 72°, was obtained in the C_6H_6 eluates (yield 900 mg, 30% from the ester). ^{1}H NMR; ($CDCl_3$) δ: 0.92 [*d*, J = 6.5 Hz, H-3′, H-4′ (merged with H-10)], 1.05–1.50 (*m*, H-6–H-9), 6.05 (*m*, H-4, H-5), *ca* 7.10 (*m*, H-3), 5.72 (*d*, J = 14.9 Hz, H-2), 5.55 (*br s*, –NH–), 3.13 (*t*, J = 7.1 Hz, H-1′), *ca* 1.65 (*m*, H-2′).

N-*Isobutyl*-4, 5-*epoxy*-2(E)-*decadienamide* (**3**), *m*-Chloroperbenzoic acid (400 mg, 2.3 mmol) in dry CH_2Cl_2 (10 ml) was added to a soln of **2a** (500 mg, 2.3 mmol) in dry CH_2Cl_2 (25 ml) with stirring. After 16 hr the ppted *m*-chlorobenzoic acid was filtered off. Et_2O (*ca* 50 ml) was added and the mixture was washed with 3% NaOH and then H_2O. The organic layer was dried and concd. The product was finally crystallized from petrol as shining crystals (350 mg, 65%), mp 152–153°. IR ν_{max}^{KBr} cm^{-1}: 3300 (*br*), N–H; 1642, C=C; 1603, C=O; 1248, epoxide; 985, *trans* double bond. ^{1}H NMR ($CDCl_3$) δ: 0.91 [*d*, J = 6.1 Hz (merged with H-10), H-3′, H-4′], 1.05–1.65 (*m*, H-6–H-9), 2.83 (*d* of *t*, J = 5.0, 2.1 Hz, H-5), *ca* 3.15 (*m*, H-4), 6.60 (*dd*, J = 16, 6 Hz, H-3), 6.06, (*d*, J = 16 Hz, H-2), 5.95 (*br s*, –NH–), 3.12 (*t*, J = 6.4 Hz; H-1′), *ca* 1.70 (*m*, H-2′).

(±)*Erythro*-N-*isobutyl*-4, 5-*dihydroxy*-2(E)-*decenamide* (**1**). Compound **3** (300 mg, 1.25 mmol) in THF (*ca* 48 ml) was cooled to 10°. *Ca* 3 ml 30% $HClO_4$ soln was added and the mixture kept at 15° for 15 hr. The solvent was removed, H_2O (*ca* 50 ml) added and the mixture extracted with Et_2O. The Et_2O extract was washed with $NaHCO_3$ soln and then H_2O and dried. Upon concn the extract gave the product (160 mg, 50%) as a crystalline solid (Me_2CO–petrol), mp 122°. IR ν_{max}^{KBr} cm^{-1}: 3300 (*br*), N–H and O–H; 1670, C=C; 1630, C=O; 1082 and 1041, C–O; 981, *trans* double bond. ^{1}H NMR, (d_6-Me_2CO) δ: 0.83 [*d*, J = 6.6 Hz (merged with H-10), H-3′, H-4′], 1.00–1.50 (*m*, H-6–H-9), *ca* 3.52 (*m*, H-5 and OH-5), 4.02 (H-4 and OH-4), 6.76 (*dd*, J = 15.4, 5.2 Hz, H-3), 6.06 (*d*, J = 15.4 Hz, H-2), 7.14 (*br s*, NH), 2.99 (*t*, J = 6.4 Hz; H-1′) *ca* 1.60 (*m*, H-2′). ^{13}C NMR (d_6-DMSO) δ: 13.96 (13.97), C-10; 22.17 (22.17), C-9; 31.51 (31.50), C-8; 24.96 (24.96), C-7; 32.66 (32.65), C-6; 73.48 (73.48), C-5; 73.79 (73.81), C-4; 143.29 (143.29), C-3; 123.81 (123.82), C-2; 165.03 (165.06), C-1; 46.13 (46.14), C-1′; 28.15 (28.14), C-2′; 20.18 (20.18), C-3′, C-4′. Values given in parentheses are those for the corresponding carbons of the natural sample recorded at the same concn and with the same machine.

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ONTOGENETIC VARIATIONS IN C_{17} HYDROCARBON COMPOSITION IN ROOT OIL OF *CIRSIUM JAPONICUM*

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Key Word Index—*Cirsium japonicum*; Compositae; root oil; *n*-C_{17}-hydrocarbons.

Abstract—Large decreases occur in the amounts of four unsaturated hydrocarbons present in the root oil of *Cirsium japonicum* during the months of February and March. These decreases are correlated with the onset of flowering.

Dihydroaplotaxene **2**, tetrahydroaplotaxene **3** and *cis*-8,9-epoxyheptadeca-1-en-11,13-diyn-10-ol from *Cirsium japonicum* have been the subject of previous studies [1, 2]. The *n*-C_{17}-hydrocarbons, aplotaxene **1**, **2**, **3** and hexahydroaplotaxene **4**, are the main components of the root oil. In order to study the changes in hydrocarbon composition during growth, root oils were prepared monthly and the contents of **1**–**4** determined by GC.