STRUCTURE AND SYNTHESIS OF (−)-ANABELLAMIDE. A NEW PHENYLALANINE DERIVED ESTER AMIDE FROM *ANAPHALIS SUBUMBELLATA*: OCCURRENCE OF 4'-HYDROXYDEHYDROKAWAIN

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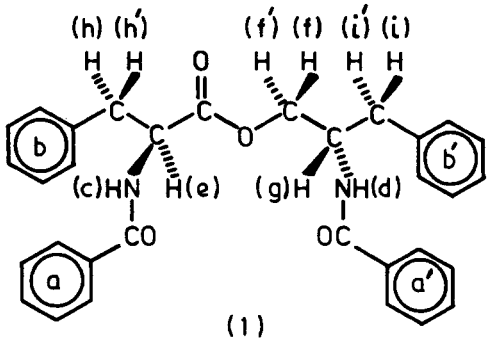
ABSTRACT.—A new ester amide designated (−)-anabellamide was isolated along with 4'-hydroxydehydrokawain (**2**) and β-sitosterol-β-D-glucoside from the whole plant of *Anaphalis subumbellata*. (−)-Anabellamide has been shown to be **1**, the (*S*)-*N*-benzoylphenylalaninyl ester of (*S*)-*N*-benzoylphenylalanine on the basis of its spectrometric studies and its synthesis from (*S*)-(−)-phenylalanine.

Under a research program of the chemical investigation of Indian *Anaphalis* species (Fam: Asteraceae), we studied the plants *A. adnata* DC (1) and *A. contorta* Hook f (2). We now report the isolation and characterization of the constituents of *A. subumbellata* Clarke, a herb growing in the Sikkim Himalayas (3).

DISCUSSION

Extensive chromatography over silica gel of the petroleum ether, chloroform, and ethanol extracts of the whole plant of *A. subumbellata* afforded a new phenylalanine-derived ester amide designated (−)-anabellamide (**1**) together with 4'-hydroxydehydrokawain (**2**) and β-sitosterol-β-D-glucoside. (−)-Anabellamide, mp 202°, $C_{32}H_{30}N_2O_4$ (M^+ 506.2174), $[\alpha]^{30}D$ −24.3° ($CHCl_3$), has been assigned the structure **1** from spectral and synthetic evidence (4,5).



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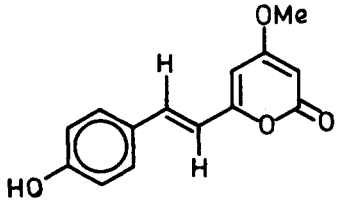

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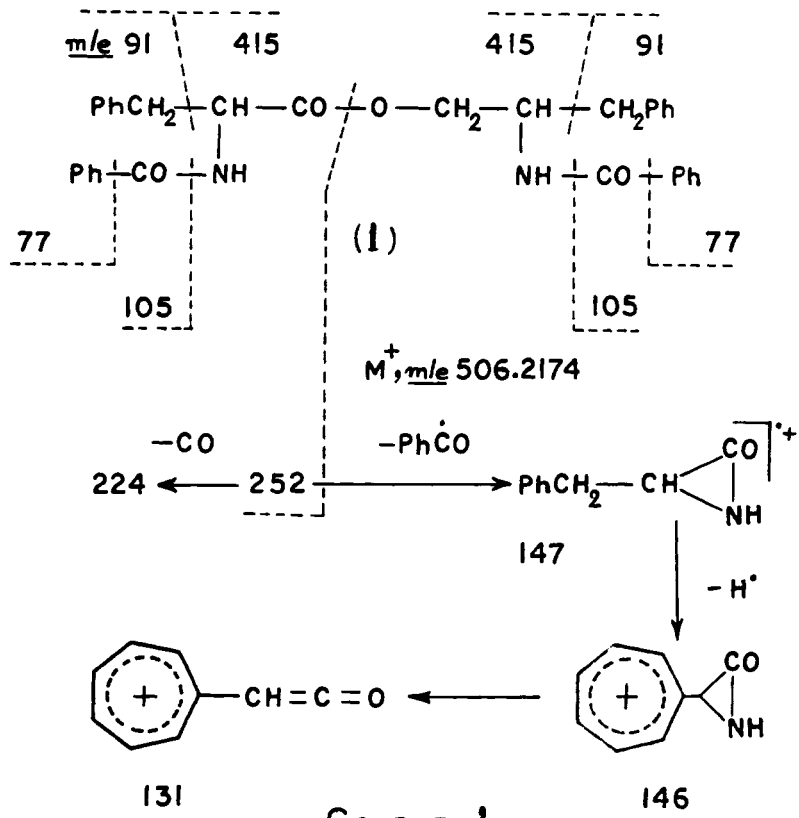
The ir spectrum of (−)-anabellamide exhibited characteristic bands at 3320 and 1640 cm^{-1} (NH-CO), 1750 and 1212 cm^{-1} (–CO–OR), and 750 and 695 cm^{-1} (unsubstituted phenyl). The structure **1** for anabellamide, without the stereochemistry at the chiral centers, could be deduced from careful analyses of its mass spectral fragmentation pattern (scheme 1) and the 80 MHz ^1H nmr spectrum showing the following signals: δ 7.55–7.80 [4H, *m, ortho* protons of COC_6H_5 (a

and a') groups], 7.30–7.51 [6H, *m*, *meta* and *para* protons of COC_6H_5 (a and a') groups], 7.20 [10H, br. *s*, aromatic protons of $CH_2C_6H_5$ (b and b') groups], 6.66 (1H, *d*, $J=6.8$ Hz, exchangeable with D_2O, c proton), 6.64 (1H, *d*, $J=6.8$ Hz, exchangeable with D_2O, d proton), 4.92 (1H, *q*, $J=6.8$ Hz, changed to a *t*, $J=6.8$ Hz, on D_2O shaking, e proton), 4.35–4.73 (2H, *m*, f and f' protons), 3.60–4.15 (1H, *m*, multiplicity decreased on D_2O shaking, g proton), 3.33 (1H, *dd*, $J=13.5$ and 6.8 Hz, h proton), 3.15 (1H, *dd*, $J=13.5$ and 6.8 Hz, h' proton), 3.05 (1H, *dd*, $J=14$ and 6.5 Hz, i proton) and 2.83 (1H, *dd*, $J=14$ and 8 Hz, i' proton).

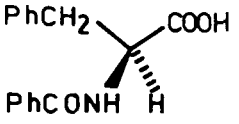


SCHEME 1

Due to a paucity of material, (−)-anabellamide could not be subjected to ester hydrolysis or to any other reaction. The structure and the absolute configuration of the chiral centers were confirmed by its synthesis: (*S*)-(+)-*N*-benzoyl phenylalanine (**3**), mp 142°, $[\alpha]^{30}D+18.5°$ (CH_3OH), was esterified with (*S*)-(−)-*N*-benzoylphenylalaninol (**4**), mp 178°, $[\alpha]^{30}D-13.3°$ ($CHCl_3$), in the presence of dicyclohexylcarbodiimide to yield (**1**). Starting from (*S*)-(−)-phenylalanine, the alcohol (**4**) was synthesized by two routes:—(i) *via* (**5**) and (**6**) and (ii) *via* (**7**) and (**8**).

The biogenesis of this modified phenylalanine-derived ester (**1**) is quite interesting. It is probably formed in the plant cells by specific enzyme catalyzed esterification of two (*S*)-phenylalanine units (**3**) and (**4**) prior *N*-benzoylation of both units thus inhibiting the formation of the modified phenylalanine dipeptide aurantiamide (6) or its acetate (6–8).

4'-Hydroxydehydrokawain (**2**), isolated in 0.02% yield from the chloroform extract of the plant, was identified from its spectral data (uv, ir, ^1H nmr and mass spectra) and by direct comparison with an authentic sample (1). The ethanol



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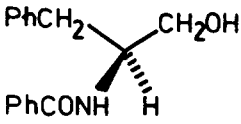

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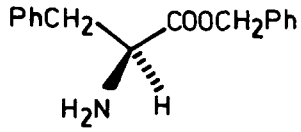

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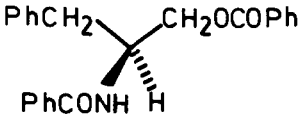

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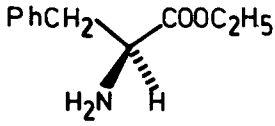

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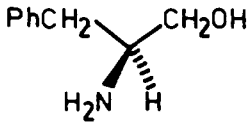

(8)

extract furnished β-sitosterol-β-D-glucoside (yield 0.001%), identified again by direct comparison.

EXPERIMENTAL[1]

PLANT MATERIAL.—The whole plant of *Anaphalis subumbellata* was collected near Darjeeling, West Bengal, in May, 1979.

EXTRACTION.—Dried and powdered whole plant (1.5 kg) of *A. subumbellata* was extracted with a soxhlet apparatus successively with petroleum ether (60–80°) and chloroform for 40 hr., in each case, and then extracted with ethanol at room temperature. The concentrates of the different extracts were chromatographed separately over silica gel.

(−)-ANABELLAMIDE (**1**).—Chromatography of the concentrate of the petroleum ether extract afforded a light brown solid from the chloroform-methanol (19:1) eluate fractions. Rechromatography of this solid over silica gel with chloroform-methanol (19:1) as eluent furnished (−)-anabellamide (**1**), which crystallized from chloroform-methanol in very fine colorless needles (15 mg), mp 202°.

4'-HYDROXYDEHYDROKAWAIN (**2**).—The concentrate of the chloroform extract was chromatographed. The brown solid obtained from the chloroform-methanol (19:1) eluate fractions on rechromatography over silica gel followed by crystallization from methanol-chloroform afforded a greenish yellow compound, 4'-hydroxydehydrokawain (**2**) (300 mg), mp 265°, M^+ 244; acetate, mp 182°; methyl ether (with diazomethane) mp 146°.

β-SITOSTEROL-β-*D*-GLUCOSIDE.—Chromatography of the concentrate of the ethanol extract afforded from the chloroform-methanol (4:1) eluate fractions β-sitosterol-β-D-glucoside as a colorless amorphous solid (15 mg), mp 265°; acetate, mp 168°, $[\alpha]^{30}D$ −27° ($CHCl_3$).

SYNTHESIS OF (−)-(*S*)-*N*-BENZOYLPHENYLALANINOL (**4**).—

METHOD—1

BENZYL ESTER (**5**) OF (*S*)-(−)-PHENYLALANINE.—For one hour, dry hydrogen chloride gas was passed through a well-stirred suspension of (*S*)-(−)-phenylalanine, $[\alpha]^{30}D$ −35° (H_2O),

[1]Melting points are uncorrected. ^1H nmr (TMS int. standard), uv, ir and mass spectra were recorded on Varian Associates CFT-20 (80 MHz), Varian Techtron series 634, Beckmann IR 20 and Jeol JMS T 100 spectrometers, respectively. The optical rotations were measured with a Perkin-Elmer 241 polarimeter. In all chromatography experiments, silica gel (Gouri Chemical, Calcutta, 100–200 mesh) was used as adsorbent.

(1g) in benzyl alcohol (20 ml) (9). The reaction mixture was then filtered, and the solid was crystallized from methanol-ether; the benzyl ester of (*S*)-(−)-phenylalanine was obtained as colorless needles (1.2 g, yield 77.6%) mp 205°, $[\alpha]^{30}D$ −10.5° (MeOH); ir, νmax (KBr) 1738 cm^{-1} (ester C=O).

(*S*)-(−)-*N,O*-DIBENZOYLPHENYLALANINOL (**6**).—To a suspension of the benzyl ester of (*S*)-(−)-phenylalanine (800 mg) in dry tetrahydrofuran a suspension of lithium aluminum hydride in the same solvent was added, and the reaction mixture was refluxed for 4 hr. Usual work up of the mixture gave an oily substance which, when benzoylated (benzoyl chloride/10% sodium hydroxide soln.), afforded (*S*)-(−)-*N,O*-dibenzoylphenylalaninol (**6**), crystallizing from chloroform in colorless needles (600 mg, yield 53%), mp 165°, $[\alpha]^{30}D$ −4° ($CHCl_3$); ir, νmax (KBr) 3320 (-N-H), 1720, 1710 (ester C=O) and 1635 cm^{-1} (amide C=O), analysis: Calcd for $C_{23}H_{21}NO_3$: C, 76.86%; H, 5.89%; N, 3.90%. Found: C, 76.36%; H, 5.81%, N, 3.67%.

(*S*)-(−)-*N*-BENZOYLPHYNYLALANINOL (**4**).—(*S*)-(−)-*N,O*-Dibenzoylphenylalaninol (100 mg) was refluxed with 2.5 (N) ethanolic HCl (20 ml) for 1.5 hr. The reaction mixture was diluted with water and extracted with chloroform. Removal of chloroform followed by crystallization of the residue from chloroform-petroleum ether furnished (*S*)-(−)-N-benzoylphenylalaninol (**4**), as fine colorless needles (17.5 mg, 29.6% yield), mp 178°, $[\alpha]^{30}D$ −13.3° ($CHCl_3$); ir, νmax (KBr) 3400–3200 (O-H and N-H) and 1638 (amide C=O); Analysis: Calcd for $C_{16}H_{17}NO_2$: C, 75.27%; H, 6.71%; N, 5.49%. Found: C, 74.98%; H, 6.82%; N, 5.29%.

METHOD—2

ETHYL ESTER (**7**) OF (*S*)-(−)-PHENYLALANINE.—Ethyl ester of (*S*)-(−)-phenylalanine (**7**) was obtained as a colorless liquid (bp 142°/10 mm) from phenylalanine, absolute alcohol and thionyl chloride according to the method of Yamada *et. al.* (10). When this ester was kept at room temperature for 2 days, a colorless crystalline compound separated. This solid was collected and was found to be 2,5-dibenzyl 3,6-diketopiperizine, $C_{18}H_{18}N_2O_2$, mp 295° (d), $[\alpha]^{30}D$ −70° (MeOH), from its ir and mass spectral features; ir, νmax (KBr) 3200 (N-H) and 1650 (amide CO); ms: important ion peaks at *m/e* (%) 294 (M^+, 62.6), 203 (29.9), 175 (38.2) and 91 (100).

(*S*)-(−)-PHENYLALANINOL (**8**).—The ethyl ester (**7**) of (*S*)-(−)-phenylalanine was reduced with sodium borohydride and absolute ethanol to (*S*)-(−)-phenylalaninol by the method of Seki *et. al.* (11). (−)-Phenylalaninol crystallized from ether as colorless needles, mp 93°, $[\alpha]^{30}D$ −23.8° (EtOH).

(*S*)-(−)-*N*-BENZOYLPHENYLALANINOL (**4**).—To a solution of (*S*)-(−)-phenylalaninol (200 mg) in a mixture of acetone and 10% potassium hydroxide solution (1:4) (50 ml) benzoyl chloride was added and shaken. The separated solid was kept suspended in the reaction mixture for 24 hr. The solid was removed by filtration and crystallized from chloroform-petroleum ether to afford (*S*)-(−)-*N*-benzoylphenylalaninol (210 mg), mp 178°.

(*S*)-(+)-*N*-BENZOYLPHENYLALANINE (**3**).—(*S*)-(−)-Phenylalanine was benzoylated as usual to give (*S*)-(+)-*N*-benzoylphenylalanine (**3**), crystallizing from hot water, mp 142–3°, $[\alpha]^{30}D$ +18.5° (CH_3OH); lit. (12), mp 142–3°, $[\alpha]D$+19.5°.

ESTERIFICATION OF (*S*)-(−)-*N*-BENZOYLPHENYLALANINOL (**4**) WITH (*S*)-(+)-*N*-BENZOYLPHENYLALANINE (**3**): SYNTHESIS OF (−)-ANABELLAMIDE (**1**).—A mixture of the acid (**3**) (50 mg), the alcohol (**4**) (50 mg) and dicyclohexylcarbodiimide (50 mg) was refluxed in tetrahydrofuran in an atmosphere of nitrogen for 8 hr. The solvent was removed and the residue was treated with cold dilute hydrochloric acid and extracted with chloroform. The concentrate of the chloroform extract was chromatographed over silica gel. The chloroform-methanol (19:1) eluate fractions afforded (−)-anabellamide (21 mg), mp 202°, $[\alpha]^{28}D$ −20.6° ($CHCl_3$), identical in all respects (mmp, co-tlc and superimposable ir spectra) with the natural compound.

ACKNOWLEDGMENTS

The authors wish to thank Mr. D. Dance (Stirling, UK) for mass spectral data and the University Grants Commission, New Delhi, for financial support.

Received 5 April 1982

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