All intermediates and products were controlled by tlc on silica. Protected compounds were run in a suitable mixture of MeOH and chloroform. Deprotected compounds were run in *n*-BuOH–HOAc–H_2O, 7:1:2. Spots were detected by the *tert*-butyl hypochlorite–starch–iodide method.[7] All compounds reported here were essentially homogeneous on tlc.

The *N*-carbobenzoxy-β-benzyl aspartate was prepared in our pilot plant by T. J. Telinski, L. J. Sacco, and R. Shubart. The crude product was crystallized from 3 parts of HOAc and 6 parts of H_2O to give homogeneous material, mp 110–111° (lit.[8] 107–109°). It was not possible to achieve this melting point using other solvent combinations.

Most amines were commercially available. It was necessary to synthesize the following: 1-ethylphenethylamine,[9] 2-methylphenethylamine,[10] 1-methyl-2-phenoxyethylamine,[11] 2-phenoxyethylamine,[12] 1-hydroxymethylphenethylamine (phenylalaninol).[13] 1-hydroxymethyl-2-(4-hydroxyphenyl)ethylamine (tyrosinol),[13] 1-methyl-2-(4-fluorophenyl)ethylamine,[14] 2-(4-fluorophenyl)-ethylamine,[15] 1-methyl-2-(2-furyl)ethylamine,[15] 1-methyl-2-cyclohexylethylamine,[16] *N*,1-dimethyl-2-cyclohexylethylamine,[16] 1-methyl-3-ethoxypropylamine,[17] 1-methyl-2-(4-methanesulfonylaminophenyl)ethylamine.[18]

Amides were prepared by the *p*-nitrophenyl ester[19] or mixed anhydride procedure.[20] Hydrogenations of protected amides were carried out in either 90% HOAc or MeOH over 10% by weight of Pd at room temperature and up to 4 atm pressure.

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12-Carboxyeudesma-3,11(13)-diene. A Novel Sesquiterpenic Acid with a Narrow Antifungal Spectrum[1]

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Received May 28, 1970

An antifungal sesquiterpenic acid, 12-carboxyeudesma-3,11(13)-diene, has been isolated from the leaves of a Mediterranean herb *Inula viscosa* Ait. *In vitro* evaluation of its potential antifungal and antibacterial properties revealed a selective antidermatophytic activity and scarcely any activity against Gram-positive and Gram-negative bacteria and mycobacteria. The presence of a free CO_2H and two olefinic bonds in the molecule is essential for its antidermatophytic activity. The *in vitro* antidermatophytic efficacy of 12-carboxyeudesma-3,11(13)-diene is very low, in the order of 1 mg/ml; however, when tested orally in mice it showed a favorable therapeutic index in the order of 10^2. These findings warrant further investigation of compounds structurally related to 12-carboxyeudesma-3,11(13)-diene, as potential antidermatophytic agents.

The isolation and partial chemical and pharmacological characterisation of a novel sesquiterpene 12-carboxyeudesma-3,11(13)-diene from the dried leaves of *Inula viscosa* Ait. are reported.

I. viscosa Ait. is a wild-growing perennial plant belonging to the family Compositae and is found abundantly along the Mediterranean shores. Folk medical tradition has ascribed antiinflammatory and antipyretic properties to this plant[2] and therefore, a search for potential medicinal products present in it was carried out. In the course of this search, attention was focussed on the selective *in vitro* antidermatophytic activity of petroleum ether extracts of the dried leaves. Consequently, the active extracts were fractionated, an active component was isolated, and its structure elucidated. To our knowledge, 12-carboxyeudesma-3,11(13)-diene is the first sesquiterpene exhibiting a specific antifungal activity.

Chemistry.—12-Carboxyeudesma-3,11(13)-diene (I) was obtained as a colorless, low-melting point compound from the petroleum ether extracts of the dried leaves of *I. viscosa* following alkaline extraction and column chromatography in a total yield of *ca.* 0.5%. Analytical results and molecular weight determination ($M^+ = 234$) by mass spectrometry established the molecular formula of I as $C_{15}H_{22}O_2$. Further support for this molecular formula was obtained from the analytical results on the cyclohexylamine salt of I, $C_{21}H_{35}NO_2$(I + $C_6H_{13}N$). The presence of an α,β-unsaturated CO_2H in I was evident from its ir spectrum as well as its uv spectrum. The nmr spectrum of I clearly indicated the presence of the following groups: $CH_3C<$, $CH_3C{=}CH$, $>C{=}CH_2$, and $-CO_2H$.

The analytical and spectral data of I presented so far strongly suggested a bicyclic sesquiterpene structure. Further support for this structure was obtained by Se dehydrogenation of I which gave, in *ca.* 25% yield, a naphthalenic hydrocarbon identified as 1-methyl-7-ethylnaphthalene (II) a compound identical in every respect with that obtained by dehydrogenation of isoalantolactone (IV). The formation of II accounts for 13 of the 15 C atoms present in I. Of the remaining two, one can be accounted for as the angular Me group which is eliminated as CH_4 during dehydrogenation, and the other as the CO_2H which is lost due to decar-

(1) This work was supported by a family fund of Dr. H. W. Rudel, New York, N. Y.
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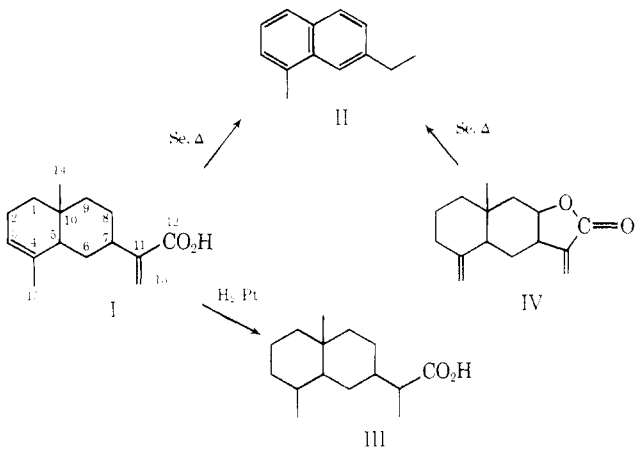
boxylation. Total hydrogenation of I required 2 moles of H_2 and gave the tetrahydro derivative 12-carboxyeudesmane (III), an oily product. The ir spectrum and the specific rotation of the Me ester of III were in good agreement with those reported for the Me ester of tetrahydrocostic acid.[3] The structure of I was thus established as 12-carboxyeudesma-3,11(13)-diene, an eudesmane structure found in sesquiterpenes, and its partial stereochemistry at C_5, C_7, and C_{10} determined.[4]



It is interesting to note the ease with which I, upon esterification with excess CH_2N_2, adds CH_2N_2 to the olefinic bond conjugated with the ester group. This course of reaction is reflected in the nmr spectrum by the disappearance of the signals at δ 5.66 s and 6.32 s and the appearance of a multiplet resembling a double doublet at δ 4.54. Upon gentle heating, the CH_2N_2 adduct loses N_2, the nmr spectrum of the resulting product shows signals characteristic of cyclopropane hydrogens and the ir spectrum shows a strong band at 1,745 cm^{-1}—indicative of a saturated ester CO. The loss of N_2 from the CH_2N_2 adduct is also evident from its mass spectrum which gives molecular peak at M^+ 262 (addition of CH_2 group across the olefinic bond conjugated with the Me ester of I: 234 + 14 + 14), accompanied by peaks at M^+ 247 (loss of Me) and M^+ 231 (loss of MeO).

The crude alkaline extract of I contains, most likely, also the isomeric compound of I — costic acid (V),[3] as can be judged from the nmr spectrum which shows a signal at δ 0.76 s due to an angular Me, and signals at δ 4.40 and 4.70 due to the additional terminal CH_2.

Biological Results. Antifungal Studies.—For establishing the antifungal spectrum of I, 8 species of fungi were used which are representative of the growth patterns of a number of pathogenic fungi encountered by man. The species used were *Trichophyton mentagrophytes, T. rubrum, T. tonsurans, T. violaceum, Epidermophyton floccosum, Microsporum gypseum, Cryptococcus neoformans, and Candida albicans.* The fungi were fresh isolates from the skin, skin appendages, or mucous membranes of patients attending the Department of Dermatology of the Beilinson Hospital. The strains were maintained in serial culture by standard techniques. The sensitivity of the various fungi to I was tested by the cylinder-plate diffusion method. Plates of minimal media (Sabouraud's dextrose agar) were seeded with spores or macerated mycelia from 10-day old cultures of each organism. Aliquots (0.1 ml) of concentrated Me_2CO solution or aq suspension of I (5 mg/ml) were pipetted into cylinders placed upon the seeded plates. Within the limits of this test, the sensitivity of a particular strain to I was indicated by a zone of growth inhibition following a 2-day incubation at 30° in the case of *C. albicans* and *Cryptococcus neoformans* and a 7-day incubation for the other species. Me_2CO in itself had no effect on fungal growth. I was found to be active against dermatophytes (*T. mentagrophytes, T. rubrum, T. tonsurans, T. violaceum, E. floccosum, M. gypseum*), but essentially inactive against *C. albicans* and *Cr. neoformans.* The Me ester of I as well as its tetrahydro derivative (III) were devoid of any antifungal activity. The presence of human serum in the medium (50%), completely abolished the antifungal activity of I. For quantitative evaluation of the antifungal activity of I, tolnaftate—a clinically established antifungal agent used for topical treatment of dermatophytoses was assayed concurrently. Under the same experimental conditions the minimal inhibitory concentrations of tolnaftate against dermatophytes were in the order of micrograms per milliliter, whereas those of I were in the order of milligrams per milliliter.

Antibacterial Studies.—The potential antimicrobial properties of I were evaluated *in vitro* according to standard techniques.[5] The following Gram-positive and Gram-negative bacteria and mycobacteria were tested and found to be unaffected by I at a concentration level of 5 mg/ml: *Staphylococcus aureus* coagulase pos., *Diplococcus pneumoniae, Bacillus anthracis, Haemophylus influenzae, Escherichia coli, Klebsiella spp., Proteus mirabilis, Pseudomonas aeruginosa, Salmonella paratyphi C, Shigella flexneri, Brucella abortus, Mycobacterium tuberculosis,* and *M. fortuitum.*

Acute Toxicity Studies.—The ip LD_{50} of I (in aq soln) in mice (18–22 g male albino mice) was 200 ± 25 mg/kg, within a 9-day period. The oral LD_{50} of I in mice, under the same experimental conditions, was 1000 ± 100 mg/kg. Death was caused by respiratory depression of central origin with developing cyanosis.

Effect on Smooth Muscle.—Results obtained with the isolated ileum of guinea pig indicated that I possesses neither spasmogenic nor atropine-like, antihistaminic, antiserotonin, or ganglion-blocking properties.

Experimental Section[6]

Isolation and Characterization of 12-Carboxyeudesma-3,11(13)-diene (I).—The powder of the air-dried leaves of *I. viscosa* Ait. (100 g) was extracted with boiling pet. ether (bp 60–80°) for 24 hr in a continuous extraction apparatus. The petroleum extract was evaporated to dryness under vacuum and the oily colored residue (*ca.* 7 g) was dissolved in Et_2O and extracted with 0.5 *N* NaOH. The oily, colored acid fraction (*ca.* 1.6 g) was chromato-

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(6) Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Ir spectra were recorded on a Perkin-Elmer Infracord Model 337 spectrophotometer equipped with a NaCl prism. Optical rotation measurements were carried out in $CHCl_3$. Nmr spectra were taken on a Varian HA-100 spectrometer for 5–10% solns in $CDCl_3$ containing TMS. Mass spectra were taken with an Atlas CH_4 instrument, the samples being introduced directly into the ion source through a vacuum lock, electron energy 70 eV, electron current 20 μA, source temp <120°, secondary electron multiplier as the detector. Elemental analyses were performed by the microanalytical laboratory of the Wiezmann Institute of Science.

graphed on a silica gel (Merck 7734) column, and the active component, 12-carboxyeudesma-3,11(13)-diene, was eluted with $CHCl_3$–pet ether (1.5:1). I (*ca.* 500 mg) was obtained as a colorless, low melting point compound which migrated as a single spot on tlc, and could not be crystallized: ν_{max} ($CHCl_3$) 2,900, 2,830(v.s), 1.690 (v.s), 1,615 (s) (>C=C<CO_2H), 1,435(s), 1,365(m), 968 (v.s) cm^{-1}; λ_{max} 210 mμ (ε 5,500); $[\alpha]^{25}D$ +10° (*c* 0.8, $CHCl_3$); nmr, δ 0.82 s (3 H) (C_{10}-CH_3), 1.62 broadened singlet due to allylic coupling (3 H) (C_4-CH_3), δ 5.31 m (1 H) (C_3-H), 5.66 s (1 H) and 6.32 s (1 H) (>C=CH_2) and 8.25 (1 H) (—CO_2**H**).

Anal. ($C_{15}H_{22}O_2$) C, 76.88; H, 9.46; mol wt, 234; found, C, 76.80; H, 9.34; M^+ 234.

The cyclohexylamine salt of I was prepared by treating I (500 mg) dissolved in butanone (10 ml), with cyclohexylamine (250 mg). The cyclohexylamine salt which separated at room temperature as a crystalline mass was crystallized once from butanone and once from Me_2CO to give colorless needles, mp 152–154°.

Anal. ($C_{21}H_{35}NO_2$) C, 75.63; H, 10.58; N, 4.20; found, C, 75.63; H, 10.65; N, 4.17.

Tetrahydro Derivative of I: 12-Carboxyeudesmane (III).—I (200 mg) was dissolved in EtOH (10 ml) and hydrogenated in the presence of Adams catalyst (PtO_2) at room temperature and atmospheric pressure. The product (III) was isolated in the usual manner, as a colorless, low-melting point compound: ν_{max} (CCl_4) 3,600 (w), 2,960, 2,910, 2,855 (s), 1,710 (s), 1,460 (m), 1,390 (m) cm^{-1}.

The Me ester, obtained as an oily colorless compound, by treating III with excess CH_2N_2, showed identical data with those reported for the Me ester of tetrahydrocostic acid[3,4]: ν_{max} (neat) 2,900 (s), 1,730 (s), 1,453, 1,380 (m), 750 (s) cm^{-1}; $[\alpha]^{25}D$ + 20° (*c* 1.0, $CHCl_3$); bp 130° (0.4 mm).

Se Dehydrogenation of I.—The acid (300 mg) was heated with Se (500 mg) at 260° under N_2 for 15 hr. The product, 1-methyl-7-ethyl naphthalene, was chromatographed on alumina column (grade I) and eluted with pet ether. The uv, the ir, and the nmr spectra of 1-methyl-7-ethyl naphthalene were identical with those obtained from the dehydrogenation product of isoalantolactone: nmr, δ 1.34 t, *J* = 7 cps, (3 H) due to the Me group of 7-Et, δ 2.66 s (3 H), due to 1-Me, 2.83 q, *J* = 7 cps, (2 H) due to the two protons of CH_2 of 7-Et.

Acknowledgment.—The authors wish to thank Mr. Gabriel Spitz for his technical assistance.

Notes

Acyl Derivatives of 5-Hydroxy-6,7-benzomorphans. Prodine Congeners

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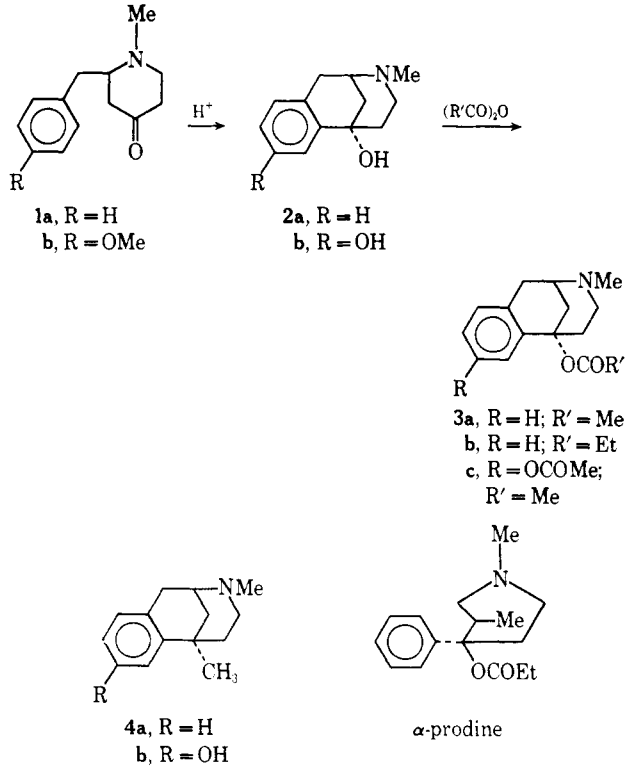
Received June 22, 1970

The synthesis of 4-piperidones (**1a,b**) from 4-methoxypyridine[2a,b] made available possible precursors of 5-hydroxy-6,7-benzomorphans (**2a,b**) acyl derivatives of which may be considered "hybrids" of the prodines[3] and 5-alkyl-6,7-benzomorphans (**4**).[4a,b] In this note are described the acid cyclization of **1a,b** to **2a,b** and some pharmacologic actions of **2a,b** and a few acyl derivatives thereof.

2-Benzyl-1-methyl-4-piperidone (**1a**)[2a] and boiling 48% HBr gave 5-hydroxy-2-methyl-6,7-benzomorphan (**2a**) in 84% yield. No 5-bromide was detected in the reaction mixture. The OH group of **2a** could not be tosylated or replaced by Cl ($SOCl_2$) or H (HI-P). In cyclizing the *p*-methoxy analog **1b** to 2′,5-dihydroxy-2-methyl-6,7-benzomorphan (**2b**), polyphosphoric acid[5] was superior to 48% HBr. Acyl derivatives **3a,b,c** were prepared from **2a,b** and the appropriate anhydride in the presence of pyridine at reflux temperature.



Pharmacology.—In Table I it is seen that the 5-OH compound **2a** has low-grade analgetic activity, and the 2′-OH relative **2b** is without effect to 100 mg/kg. Both acetylation and propionylation improve activity (compare **2a,b** with **3a,b,c**), but the degree of potency of the α-prodine or benzomorphan parents was attained only with the 5-AcO compound **3a** which is twice as potent as

(1) (a) Visiting Fellow from Tokyo, 1968–1969; (b) to whom inquiries should be addressed.

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(5) Boiling 48% HBr in this instance gave only 11% of **2b** and much tar.