Phytochemistry, 1973, Vol. 12, pp. 1755 to 1758. Pergamon Press. Printed in England.

A NEW DITERPENIC ACID AND OTHER CONSTITUENTS OF *HAPLOPAPPUS FOLIOSUS* AND *H. ANGUSTIFOLIUS**

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(*Revised Received* 11 *January* 1973. *Accepted* 23 *January* 1973)

Key Word Index—*Haplopappus foliosus*; *Haplopappus angustifolius*; Compositae; hydrocarbons; triterpenoids; stigmasterol; diterpenic acid; happlopappic acid.

Abstract—Hentriacontane, friedelan-3-one, *epi*-friedelinol, hexacosanol, stigmasterol, a new diterpenic acid and its monomethyl ester are amongst the constituents of *Haplopappus foliosus* and *H. angustifoliosus*. The new acid has been called haplopappic acid.

RESULTS AND DISCUSSION

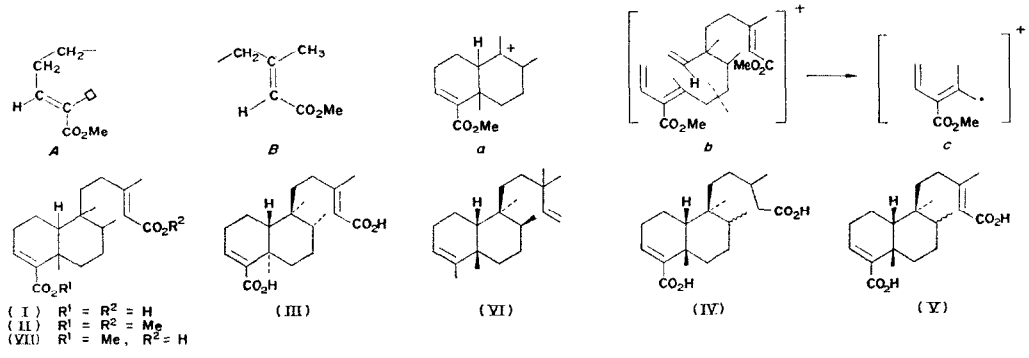
THE FATTY components of two *Haplopappus* species have been examined. Saponification of the light petroleum extract afforded a neutral and an acidic fraction. Separation of the neutral constituents by column chromatography through alumina afforded, in order of increasing polarity, hentriacontane, friedelan-3-one, epifriedelinol, hexacosanol, and stigmasterol. Similar quantities of all the neutral components, except for stigmasterol, were isolated from both *H. foliosus* and *H. angustifoliosus*; stigmasterol was not isolated from the latter species.

The acidic fraction afforded, from *H. foliosus*, an acid (I), analysed as $C_{20}H_{30}O_4$. Hydrogenation of the acid gave both a dihydro- and a tetra-hydro derivative. The acid (I) gave a negative test to tetranitromethane and had λ_{max}^{EtOH} 220 nm (ϵ 19 300), consistent with the presence of two unsaturated and conjugated acid groups. The NMR spectrum of the derived dimethyl ester (II) showed the presence of two quaternary methyl groups, one secondary methyl group and one vinylic methyl function. By appropriate decoupling experiments the presence of the part structures A and B were deduced. On the basis of these NMR results and the following MS information the ester (II) was assigned the structure shown. The MS showed important peaks at *m/e* 362 (M^+), 235, 203, 139 and 107. The ion at *m/e* 235 fits for cleavage of the side chain to give the ion (a). This ion (a) can also lose methanol to form the ion at *m/e* 203. A retro-Diels–Alder fragmentation[1] of ring A, to give the ion (b), is followed by allylic cleavage to give the ion (c) at *m/e* 107. Thus the diacid appears to be a diterpene of the clerodane type.[2] A similar dicarboxylic acid has been isolated from *Hardwickia primata* and named kolavic acid (III).[3] Comparison of the new acid isolated with kolavic acid, both as the free acid and as the methyl esters, showed subtle differences, although the basic properties were similar. Whereas the MS pattern of

* This work was supported by the Organization of American States (Grant PMC-8/1). Contribution No. 36.

[1] BIEMANN K. (1962) *Mass Spectrometry*, p. 103, McGraw-Hill, New York.
[2] FUJITA E. (1970) *Bull. Inst. Chem. Res.* (*Kyoto Univ.*) **48**, 111.
[3] MISRA, R., PANDEY, R. C. and DEV, S. (1968) *Tetrahedron Letters* 2681.

the two esters were very similar, their NMR spectra differed (Table 1). A m.m.p. of the two acids (I) and (II) was depressed. Because the NMR spectra of the two methyl esters were so similar, even in deuteriobenzene, the differences in stereochemistry between the two compounds was considered minimal. The optical rotations of the new acid and its derivatives were consistently of the opposite, but not equivalent, sign to those of kolavic acid.



Recently the diterpene cistodioic acid (IV)[4] has been isolated and on the basis of spectral evidence it has been tentatively assigned the configuration shown. MS and chromatographic comparison* with a sample of the dihydro-derivative of haplopappic acid showed that they were identical. It follows that haplopappic acid must have the structure (V), in which only the configuration about position 8 remains uncertain. Haplopappic acid is therefore another member of the *cis*-clerodane family, which also includes plathyterpol (VI).[5]

TABLE 1. NMR VALUES (τ) OF THE METHYL ESTERS*

	Position	20	17	19	16	MeO	MeO	14	3
Dimethyl haplopappate	($CDCl_3$)	9·21	9·23	8·76	7·79	6·32	6·31	4·30	3·40
	(C_6D_6)	9·34	9·34	8·76	7·91	6·74	6·57	3·74	4·32
Dimethyl kolavate	($CDCl_3$)	9·23	9·18	8·74	7·83	6·32	6·31	4·35	3·41
	(C_6D_6)	9·30	9·36	8·68	7·85	6·60	6·57	4·28	3·48

* Tetramethylsilane as internal reference. The protons occurred as singlets except for those at positions 17 (3H, *d*, *J* 6 Hz), 16 (3H, *d*, *J ca.* 1·5 Hz), 14 (1H, *m*), and 3 (1H, *t*, *J ca.* 4 Hz).

Examination of the acid fraction from *H. angustifoliosus* afforded two acids, the first of which was a monomethyl ester. Reaction with diazomethane gave the dimethyl ester (II). Attempted saponification of the monoester with ethanolic KOH proceeded only with difficulty and, for this reason, it was assigned the structure (VII). The other acid was identical to haplopappic acid (I).

* We thank Professor G. Berti for an authentic sample of cistodioic acid.

[4] BERTI, G., LIVI, O. and SEGNINI, D. (1970) *Tetrahedron Letters* 1401.

[5] KING, T. J., RODRIGO, S. and WALLWORK, S. C. (1969) *Chem. Commun.* 683.

EXPERIMENTAL

Instruments and solvents used were as described previously.[6]

Haplopappus foliosus. The dried, powdered stems and leaves (836 g) of *H. foliosus*, collected near Los Vilos, Coquimbo, Chile, were extracted with petroleum to give a dark green extract (47 g). This material was saponified with 1 N KOH to yield a neutral fraction (25 g) and an acid part (18 g). A portion of the neutral part (15 g) was chromatographed through alumina (grade III, 40 g) to yield the following components:

Hentriacontane. (1·12 g), eluted with petrol., m.p. 63–4° (from MeOH), $[\alpha]_D^{25}$ 0·00° (*c* 0·6, $CHCl_3$), ν_{max}^{film} 2933, 2877, 1471, 737, 720 cm^{-1}. Its MS showed a parent ion at *m/e* 436, corresponding to $C_{31}H_{64}$.

Friedelan-3-one. (60 mg), eluted with petrol., m.p. 228–9° (from MeOH–$CHCl_3$), $[\alpha]_D^{25}$ −15·6° (*c* 1·0, $CHCl_3$), ν_{max}^{Nujol} 1705 cm^{-1}, τ ($CDCl_3$) 7·8 (2H, broadened triplet, $-CH_2CO$), 8·5–9·5 (complex, methyl region). The MS showed *m/e* 426 (M^+), 411, 341, 302, 287, 273, 248, 246, 231, 218, 205, 191, 179, 69 (base peak), 45, 43 and 41. The fragmentation pattern and the physical constants were in agreement with those reported for friedelan-3-one; direct comparison with an authentic sample confirmed this assignment.

Epi-friedelinol. (80 mg), eluted with petrol., m.p. 270–2° (from $CHCl_3$–MeOH), $[\alpha]_D^{25}$ +13·9° (*c* 1·0, $CHCl_3$), ν_{max}^{Nujol} 3500 cm^{-1}. Oxidation of a sample of the alcohol with Jones' reagent in acetone yielded friedelan-3-one, identical with an authentic sample. On acetylation the alcohol afforded the acetate, m.p. 286–8° ($CHCl_3$–MeOH), $[\alpha]_D^{25}$ +28·7° (*c* 0·9, $CHCl_3$). Its NMR spectrum showed the presence of only one acetate group, τ 7·85, and one proton adjacent to the acetate function at 5·10. The free alcohol was identical to an authentic sample by m.p., m. m.p., TLC and IR.

Hexacosanol. (40 mg), eluted with petrol., m.p. 62° ($CHCl_3$–MeOH), $[\alpha]_D^{25}$ 0·00° (*c* 1·00, $CHCl_3$), ν_{max}^{Nujol} 3500 cm^{-1}, M^+ 382, with a fragmentation pattern typical for a normal aliphatic alcohol.[7] On acetylation a monoacetate formed, m.p. 59–60°. The MS of this showed the parent ion at *m/e* 424.

Stigmasterol. (30 mg), eluted with petrol.–C_6H_6, m.p. 170° (EtOH), $[\alpha]_D^{25}$ −49° (*c* 1·0, $CHCl_3$; λ_{max}^{EtOH} 206 nm (ϵ 5000), ν_{max}^{Nujol} 3500 cm^{-1}, *m/e* 412 (M^+), corresponding to $C_{29}H_{48}O$. Comparison of this sample with authentic stigmasterol showed them to be identical.

Acidic fraction. The acidic material (18 g) gave, after several recrystallizations, an acid (1·5 g), m.p. 249–51° (MeOH), $[\alpha]_D^{25}$ +108° (*c* 0·55, pyridine). The crude acid was purified by column chromatography on silica gel, eluting with EtOAc–acetone, to give *haplopappic acid* (I), m.p. 242–4° (EtOH), $[\alpha]_D^{25}$ +117·6° (*c* 0·51, $CHCl_3$), λ_{max}^{EtOH} 220 nm (ϵ 19 300), ν_{max}^{Nujol} 1685, 1625 cm^{-1}, τ (CF_3CO_2H) 3·30 (1H, broadened *t*) 4·41 (1H, *m*), 7·81 (3H, *s*), 8·72 (3H, *s*), 9·16 (6H, *d* and *s*). The acid gave a negative test to tetranitromethane, to $FeCl_3$ and to the Zimmermann test. The starting material was recovered unchanged after refluxing it for 2 hr in 1 N KOH, and after treatment with either peracetic acid or Br_2 (Found: C, 71·78; H, 8·80. $C_{20}H_{30}O_4$ requires: C, 71·82; H, 9·04%). A m.m.p. with authentic kolavic acid (m.p. 224–6°) showed m.p. 215–20°.

Dimethyl haplopappate. Treatment of the acid with CH_2N_2 in Et_2O gave the *ester*, distilled at 150°/0·1 mm Hg, $[\alpha]_D^{25}$ +59° (*c* 2·0, $CHCl_3$), λ_{max}^{EtOH} 220 nm (ϵ 19 300), ν_{max}^{film} 1718, 1605 cm^{-1}, τ ($CDCl_3$), see Table 1, *m/e* 360 (M^+) (6%), 347 (12), 330 (M^+–MeOH) (58), 315 (21), 302 (11), 287 (8), 283 (19), 235 (ion a) (20), 233 (22), 203 (33), 139 (ion b) (50), 107 (30) (Found: C, 72·97; H, 9·62; MeO, 16·86. $C_{22}H_{34}O_4$ requires: C, 72·89; H, 9·45; MeO, 17·12%). This ester had R_f 0·51 on SiO_2 G (solvent, 1 : 5, acetone–petrol.); dimethyl kovalate had R_f 0·82.

Reduction of haplopappic acid. The acid (26 mg) in absolute EtOH (7 ml) was reduced over 5% Pd–C to yield the *dihydro-acid*, m.p. 236° (from EtOH), $[\alpha]_D^{25}$ +91·6° (*c* 0·7, pyridine), λ_{max}^{EtOH} 216 nm (ϵ 11 800), τ (CF_3CO_2H) 4·40 (1H, broadened *s*), 7·60 (3H, *s*), 8·65 (3H, *s*), 9·15 (3H, *d*, *J* 6 Hz), 9·14 (3H, *s*) (Found: C, 71·15; H, 9·03. $C_{20}H_{32}O_4$requires: C, 71·39; H, 9·59%). *m/e* 336 (M^+, 5%), 321 (1), 318 (10), 303 (10), 285 (7), 221 (80), 203 (50), 175 (15), 139 (20), 137 (20), 125 (100), 91 (40), 69 (50), 54 (70). The MS and IR absorption curve of this compound were identical to those of an authentic sample of cistodioic acid. Comparison by TLC using multiple elution techniques, also indicated identity. The authentic sample had m.p. 255–6°, m.m.p. 250–6°. When the reduction was carried out in AcOH (5 ml) with the acid (33 mg) using Adam's catalyst (25 mg), 2 mol of H_2 were absorbed to give the *tetrahydro-acid*, m.p. 224–6° (from EtOH), $[\alpha]_D^{25}$ +37·4° (*c* 0·4, pyridine), UV end absorption only, τ (CF_3CO_2H) 7·54 (2H, *m*), 8·78 (3H, *s*), 8·92 (6H, *d*), 8·94 (3H, *s*) (Found: C, 71·17; H, 10·03. $C_{20}H_{34}O_4$ requires: C, 70·97; H, 10·13%). The tetrahydro-acid reacted with diazomethane to give an oily ester, $[\alpha]_D^{25}$ +18·1° (*c* 1·3, $CHCl_3$), ν_{max}^{film} 1745 cm^{-1}.

Haplopappus angustifoliosus. Dried and ground leaves and stems of the plant (4·9 kg), collected at the same site as *H. foliosus*, were extracted with petrol. After removal of the solvent a dark green extract was obtained (260 g). A portion (65 g) was saponified as for the previous plant and the neutral fraction (13 g)

[6] PACHECO, P., SILVA, M., SAMMES, P. G., and TYLER, T. W. (1973) *Phytochemistry* **12**, 893.

[7] BUDZIKIEWICZ, H., DJERASSI, C. and WILLIAMS, D. H. (1964) *Interpretation of Mass Spectra of Organic Compounds*, p. 28, Holden Day, San Francisco.

gave, by column chromatography through alumina the following compounds, identified as above: hentriacontane (150 mg), friedelan-3-one (80 mg), *epi*-friedelinol (130 mg), and hexacosanol (60 mg). No stigmasterol could be detected in this extract. The acid fraction, on purification by column chromatography through silica afforded two compounds by elution with EtOAc. The second component was haplopappic acid (2·53 g), m.p. 240–2°, identical by direct comparison to the material isolated from *H. foliosus*. The first fraction from the column crystallized from EtOH to give an ester (6 g), m.p. 120–2°, $[\alpha]_D^{25}$ +76° 0·8, $CHCl_3$), λ_{max}^{EtOH} 221 nm (ε 23 800), ν_{max}^{KBr} 1720, 1650 cm^{-1}. On standing the ester with an excess of diazomethane in Et_2O for a short period the dimethyl ester (II) formed, identical by spectral and TLC comparison with the authentic material. Attempted hydrolysis of the monomethyl ester with 1 N KOH for an extended period gave back mainly the starting material.

Acknowledgements—We thank Professors K. Y. Sim, University of Singapore, W. H. Hui, University of Hong Kong, and Sukh Dev, National Chemical Laboratory, Poona, for kindly providing authentic samples. We thank Dr. C. Marticorena for the botanical identifications and the Fund for Overseas Research Grants and Education, New York for financial assistance.