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ISOLATION AND IDENTIFICATION OF TRACHYLOBAN-19-OIC AND (−)-KAUR-16-EN-19-OIC ACIDS AS ANTIMICROBIAL AGENTS FROM THE PRAIRIE SUNFLOWER, *HELIANTHUS ANNUUS*

L.A. MITSCHER, G.S.R. RAO, T. VEYSOGLU, S. DRAKE, and T. HAAS

Department of Medicinal Chemistry, Kansas University, Laurence, KS 66045

Several investigators have studied *Helianthus annuus* L. (Fam. Compositae) in a search for novel antimicrobial agents. Chlorogenic acid (1), isochlorogenic acid (2), and annuithrin (3) have been so identified previously. In addition, because of its commercial importance and botanical prominence, several recent phytochemical studies of the Helianthae have also been carried out (4-6). We wish to report that the stems of *Helianthus annuus* collected in Douglas County, Kansas, in the fall of 1979-1981 were subjected to bioassay-directed fractionation (7) and gave small quantities of trachyloban-19-oic (8,9) and (−)-kaur-16-en-19-oic (9,10) acids as active antimicrobial constituents. Interestingly, the same two acids have recently been shown to be responsible for resistance of some strains of sunflower to the sunflower moth (*Homoeosoma electellum*) (11). (−)-Kaur-16-en-19-oic acid has also been isolated as an antimicrobial agent from *Croton argyrophylloides* (16) and *Minania monagasensis* (17).

EXPERIMENTAL[1]

PLANT MATERIAL.—The plants were collected in Douglas County, Kansas, under the supervision of Mr. Ralph Brooks of The Kansas Biological Survey, Lawrence, where a voucher specimen is on deposit.

EXTRACTION AND FRACTIONATION.—Air-dried and powdered stems (3 kg) were extracted by Soxhlet with ethanol to give 270 g of active extract. Solvent partition in the usual manner (7) gave 7.5 g of active polar lipids and 0.28 g of weakly active alkaloids. The polar lipids were chromatographed over silica gel using hexane-ethyl acetate (4:1). The active fractions were combined and evaporated to a solid residue which gave 0.120 g of a crystalline mixture of carboxylic acids (mp, 139-143°). This was methylated with diazomethane in the usual fashion. Despite homogeneity in 19 thin layer systems, the ^{13}C-nmr spectrum indicated that the product was a mixture of two esters in nearly equal amounts. Preparative argentation chromatography on silica gel treated with silver nitrate, using hexane-benzene (1:1) for elution (8) gave an excellent separation of two compounds (40 mg each) which proved identical in their physical and spectral properties to the methyl esters of the known diterpene acids trachloban-19-oic acid (8.9), and (−)-kaur-16-en-19-oic acid (8,9). Regeneration of the free acids confirmed the identity.

IDENTIFICATION OF TERPENE ACIDS.—Methyl trachyloban-19-oate (8-10): mp, 96-97°; ir, ($CHCl_3$) 1730, 1260, 1200, 1160 and 1150 cm^{-1}; pmr ($CDCl_3$) 3.63 (3H, s), 0.60 (m), 0.76 (3H, s), 1.12 (3H, s), 1.14 (3H, s), 1.09-2.30δ (m), etc.; ^{13}C-nmr ($CDCl_3$) 177.94, 57.03, 52.75, 51.02, 50.36, 43.74, 40.81, 39.51, 39.30, 38.66, 38.15, 30.09, 28.67, 24.25, 22.35, 21.82, 20.52, 20.37, 19.69, 18.76 and 12.34 ppm; ms, *m/z* 316 (74%, M^+), 301 (29), 284 (4.3), 274 (10), 260 (68), 257 (36), 256 (13), 245 (29), 241 (31), 201 (22), 175 (20), 161 (21), 159 (24), 147 (27), 145 (25), 134 (28), 133 (31), 131 (28), 123 (29), 121 (68), 118 (23), 107 (44), 106 (39), and 105 (100).

Methyl (−)-kaur-16-en-19-oate (8,10,12,13): mp, 70-71°; ir, ($CHCl_3$) 3070, 1720 and 1655 cm^{-1}; pmr ($CDCl_3$) 0.83 (3H, s), 1.17 (3H, s), 3.64 (3H, s), 4.76 (2H, brm.), etc.; ^{13}C-nmr ($CDCl_3$) 178.04, 155.82, 102.98, 57.10, 55.13, 51.04, 48.98, 44.23, 43.83, 43.76, 41.32, 40.80, 39.68, 39.44, 38.13, 33.11, 28.73, 21.92, 19.15, 18.39 and 15.41 ppm; ms, *m/z* 316 (36%, M^+), 301 (24), 274 (9), 273 (37), 269 (4), 258 (11), 257 (60), 256 (26), 241 (51), 240 (11), 213 (29), 201 (12), 199 (15), 187 (19), 185 (15), 175 (10), 161 (16), 159 (19), 149 (14), 148 (22), 147 (23), 145 (19), 135 (21), 134 (15), 131 (54), 129 (11), 123 (59), 122 (16), 107 (68), and 91 (100).

The pure esters were separately hydrolyzed to the parent acids by refluxing for 10 h with lithium bromide in dry dimethylformamide (14). The products were further purified by chromatography over silica gel with hexane-ethyl acetate mixture for elution and then by crystallization.

Trachyloban-19-oic acid: mp, 164-166°; ir; ($CHCl_3$) 2940, 2880, 1700, 1460, 1440 and 1310 cm^{-1}; pmr ($CDCl_3$) 0.81 (3H, s), 1.05

[1]Full details of the isolation and identification are available from the authors upon request.

TABLE 1. Antimicrobial properties of *Helianthus* agents (MIC in μg/ml) (7).

Organism	Substance				
	(1)	(2)	(3)	(4)	Streptomycin
Staphylococcus aureus ATCC 13709	6.25	12.5	i[a]	i	3.1
Escherichia coli ATCC 9637	i	i	i	i	12.5
Salmonella gallinarum ATCC 9184	i	i	i	i	25
Klebsiella pneumoniae ATCC 10031	i	i	i	i	1.56
Mycobacterium smegmatis ATCC 607	6.25	6.25	50	25	0.78
Candida albicans ATCC 10231	i	i	i	i	i

[a]i = inactive at the highest level tested (100 μg/ml).

(1) = trachyloban-19-oic acid; (3) = 1 methyl ester; (2) = (−)-kaur-16-en-19-oic acid; (4) = 2, methyl ester.

(3H, s), 1.14 (3H, s), etc.; ms, *m/z* 302 (29%, M^+), 287 (21), 246 (44), 241 (12), 231 (28), 185 (11), 175 (10), 159 (15), 148 (10), 147 (20), 145 (19), 135 (16), 134 (25), 133 (24), 131 (32), 129 (11), 123 (16), 121 (29), 119 (47), 118 (21), 117 (24), 109 (20), 107 (34), 106 (38), 105 (77), 95 (29), 94 (23), 93 (73), 92 (35), 91 (100), etc.

(−)-Kaur-16-en-19-oic acid (11.15): mp, 169-171°; ir, 2960, 2880, 1700, 1460, 1440, 1270, 1220 cm^{-1}; pmr ($CDCl_3$) 0.95 (3H, s), 1.24 (3H, s), 4.77 (2H, br. d), 1.27 to 2.24 (m); ms, *m/z* 302 (11%), 287 (16), 259 (17), 241 (25), 213 (17), 187 (13), 159 (17), 147 (19), 133 (25), 131 (58), 105 (67), and 91 (100).

Both acids were re-esterified with diazomethane to demonstrate that no irreversible change had occurred during these treatments. The close correspondence of these properties with those in the literature and the previously established occurrence of these acids in this plant leave no reasonable doubt as to their identity.

IN VITRO ANTIMICROBIAL PROPERTIES.—The compounds were tested *in vitro* against several microorganisms using the standard agar-dilution streak method and streptomycin as a comparison control (7). From the data in the table, the two acids are of nearly equivalent potency against *Staphylococcus aureus* and *Mycobacterium smegmatis* whereas the esters are substantially less active. Relative narrowness of spectrum and weakness of potency make these agents doubtful candidates for human therapy, although they probably play a significant role in protecting *Helianthus* from bacteria in the field.

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