7. Bohlmann, F., Mahanta, P. K., Natu, A. A., King, R. M. and Robinson, H. (1978) *Phytochemistry*, **17**, 471. These authors did not establish the distribution of the acetate and five carbon-ester side chain between C-3 and C-8.
8. Takahashi, T., Eto, H., Ichimura, T. and Murae, T. (1978) *Chem. Letters* 1345.
9. Pettei, M. J., Miura, I., Kubo, I. and Nakanishi, K. (1978) *Heterocycles* **11,** 471. These workers obtained **1a** in crystalline form by means of HPLC.
10. Drozdz, B., Grabarczyk, H., Samek, Z., Holub, M., Herout, V. and Šorm, F. (1972) *Collect. Czech. Chem. Commun.* **37,** 1546.
11. Holub, M. and Samek, Z. (1977) *Collect. Czech. Chem. Commun.* **42,** 1053.
12. Herz, W. and Högenauer, G. (1962) *J. Org. Chem.* **27,** 905.

Phytochemistry, 1980, Vol. 19, pp. 1237–1238. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0601-1237 $02.00/0

KAURENOID DITERPENES FROM *STACHYS LANATA*

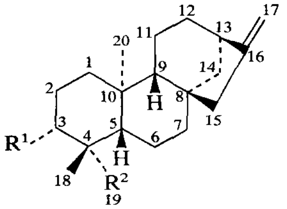
FRANCO PIOZZI,* GIUSEPPE SAVONA* and JAMES R. HANSON†

* Instituto di Chimica Organica, Universita di Palermo, Via Archirafi, Palermo, Italy;
† School of Molecular Sciences, University of Sussex, Brighton, Sussex, BN1 9QJ, U.K.

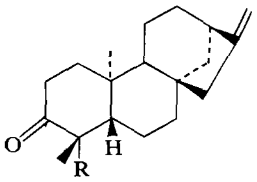
(*Received* 16 *August* 1979)

Key Word Index—*Stachys lanata;* Labiatae; *ent*-3β,19-dihydroxykaur-16-ene; *ent*-3β-hydroxykaur-16-en-19-oic acid.

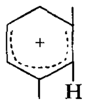
In continuation of our work on the diterpenoids of the Labiatae [1, 2], we have examined the medicinal herb, *Stachys lanata*. Extraction of the plant with acetone and chromatography afforded an acetoxy-acid, $C_{22}H_{32}O_4$ (**1**) together with a mixture of a diol, $C_{20}H_{32}O_2$ (**2**) and a hydroxy-acid, $C_{20}H_{30}O_3$ (**3**). The latter were separated by methylation and further chromatography. The ^{1}H NMR spectra of the esters **4** and **5** and the diol **2** contained resonances assigned to two tertiary methyl groups and an exocyclic methylene, suggesting that the compounds were kaurene derivatives. Oxidation of the hydroxy-ester **5** with 8 N CrO_3 gave a keto-ester **6**, whilst hydrolysis of the acetoxy-acid **1** and oxidation with 8 N CrO_3 gave a neutral nor-ketone, $C_{19}H_{30}O$ (**7**). The ^{1}H NMR spectrum of this ketone suggested that it contained a CH_3·CH group. Irradiation at δ 2.35 led to the collapse of the methyl doublet at 0.99 to a singlet. Thus, the hydroxy-acid was a β-hydroxy-acid. The multiplicity of the acetoxyl C$\underline{H}$ resonance (δ 4.52, $J = 4$ and 12 Hz) indicated that the oxygen substituent was equatorial. Comparison of the ^{13}C NMR spectra (Table 1) with the assignments for *ent*-kaur-16-ene (**8**) [3] led to the location of the hydroxyl group at C-3. The presence of oxygen functions on ring A was substantiated by a strong ion (**9**) at *m/e* 107.086 (C_8H_{11} requires 107.086) in the MS of **2**, **4** and **5**. Reduction of the methyl esters **4** and **5** afforded the diol **2** which proved to be identical to the known *ent*-3β,19-dihydroxykaur-16-ene [4, 5]. Hence, the acetoxy- and hydroxy-acids were **1** and **3** [6], respectively.



1 $R^1 = OAc$, $R^2 = CO_2H$
2 $R^1 = OH$, $R^2 = CH_2OH$
3 $R^1 = OH$ $R^2 = CO_2H$
4 $R^1 = OAc$, $R^2 = CO_2Me$
5 $R^1 = OH$, $R^2 = CO_2Me$
8 $R^1 = H$, $R^2 = Me$



6 $R = CO_2Me$
7 $R = H$



9

Whilst 19-oxidation of kaur-16-enes is common amongst the diterpenoids of the Compositae and Euphorbiaceae, 18-oxidation is more common amongst the tetracyclic diterpenoids of the Labiatae (e.g. *Sideritis* species). Stachysic acid, *ent*-6α-acetoxykaur-16-en-18-oic acid, has been obtained from *Stachys silvatica* [7].

EXPERIMENTAL

General experimental details have been described previously [1, 2].

Table 1. ^{13}C NMR spectra determined at 25.15 MHz in $CDCl_3$ with TMS as reference

Carbon atom	Compound **1**	Compound **2**	Compound **5**
1	38.8	38.7	39.5
2	24.0	27.6	28.2
3	79.0	80.6	78.3
4	47.9	42.7	48.9
5	55.2	55.8	55.0
6	21.5	20.1	21.9
7	41.0	41.3	41.2
8	43.9	43.9	43.7
9	56.4	55.8	56.4
10	39.4	39.6	39.5
11	18.6	18.3	18.6
12	33.0	33.0	33.1
13	43.8	43.9	43.7
14	39.4	38.5	39.5
15	48.7	48.8	48.7
16	155.4	155.4	155.3
17	103.3	103.1	103.2
18	23.7	22.8	23.6
19	180.3	64.3	178.6
20	15.4	18.3	15.4
	OAc 21.5 and 171.0.		OMe 51.1.

Extraction of Stachys lanata. *Stachys lanata* (1.8 kg) (obtained from M. Kottas, Vienna) was extracted with Me_2CO (10 l.) at room temp. for 3 days. The solvent was evapd and the residue chromatographed on Si gel, (Merck, deactivated with 15% H_2O). Elution with EtOAc–petrol (2:8) *gave ent-3β-acetoxykaur-16-en-19-oic acid* (**1**) (300 mg) which crystallized from EtOAc as needles, mp 255–260°. [Found: C, 72.9; H, 8.6. $C_{22}H_{32}O_4$ requires C, 73.3; H, 8.95%.] ^{1}H NMR: δ 1.05 (3H, *s*, 20-H), 1.29 (3H, *s*, 18-H) 2.08 (3H, *s*), 4.55 (1H, *dd*, $J = 4$ and 12 Hz), 4.80 (2H, *m*, 17-H). The *methyl ester*, prepared with CH_2N_2, had mp 165°, $[\alpha]_D -86°$ (*c* 1.9). [Found: M^+ 374.245·$C_{23}H_{34}O_4$ requires M^+ 374.245.] IR ν_{max} cm^{-1}: 1725 (br), 1655, 1245, 880; ^{1}H NMR: δ 0.92 (3H, *s*, 20-H), 1.21 (3H, *s*, 18-H), 2.04 (3H, *s*, OAc), 2.62 (1H, *br*), 3.65 (3H, *s*, OMe), 4.52 (1H, *dd*, $J = 4$ and 12 Hz, 3-H), 4.77 (2H, *m*, 17-H); MS *m/e* (rel. int.): 374 (8), 314 (24), 299 (18), 271 (16), 255 (30), 239 (37), 211 (40), 159 (19), 145 (23), 137 (31), 133 (23), 131 (25), 121 (36), 119 (57), 107 (61), 105 (56), 91 (100). Further elution with EtOAc–petrol (2:8) gave a mixture of the diol **2** and hydroxy-acid **3**. Methylation of the mixture with CH_2N_2 and chromatography on Si gel gave *methyl* ent-*3β-hydroxy-kaur-16-en-19-oate* (200 mg), which crystallized from petrol, mp 157°, $[\alpha]_D -107°$ (*c* 1.7). [Found: M^+ 332.238.$C_{21}H_{32}O_3$ requires 332.235.] IR ν_{max} cm^{-1}: 3450 (sh), 3400, 1690, 1655, 875; ^{1}H NMR δ 0.89 (3H, *s*, 20-H), 1.40 (3H, *s*, 18-H), 3.66 (3H, *s*, OMe), 3.12 (1H, *m*, 3-H), 4.78 (2H, *m*, 17-H); MS *m/e* (rel. int.): 332 (19), 314 (15), 289 (13), 239 (20), 211 (22), 187 (22), 145 (30), 133 (31), 131 (31), 21 (42), 119 (61), 107 (65), 105 (78), 91 (100). Subsequent fractions gave *ent*-3β,19-dihydroxykaur-16-ene, which crystallized from EtOAc–petrol as tablets, mp 190°, $[\alpha]_D -67.6°$ (*c* 1.08) (lit. [4, 5] 184–185°, $[\alpha]_D -66°$). [Found: M^+ 304.245·$C_{20}H_{32}O_2$ requires 304.240.] IR ν_{max} cm^{-1}: 3460 (sh), 3400 (br), 1655, 882; ^{1}H NMR: δ 0.99 (3H, *s*, 20-H), 1.22 (3H, *s*, 18-H), 2.66 (1H, *m*), 3.30 (1H, *d*, $J = 12$ Hz, 19-H), 3.45 (1H, *m*, 3-H), 4.15 (1H, *d*, $J = 12$ Hz, 19-H), 4.80 (2H, *m*, 17-H); MS *m/e* (rel. int.): 304 (2), 286 (26), 271 (19), 268 (16), 255 (30), 243 (30), 227 (26), 185 (28), 145 (42), 133 (40), 131 (55), 121 (52), 119 (68), 107 (78), 105 (85), 91 (100). The diacetate, prepared with Ac_2O in Py, had mp 112–114° (lit. [4, 5] 114–115°); ^{1}H NMR: δ 1.00 (3H, *s*), 1.05 (3H, *s*), 2.0 and 2.05 (each 3H, *s*), 4.12 and 4.37 (2H, AB, *q*, $J = 12$ Hz), 4.65 (1H, *m*), 4.76 (2H, *m*). The hydroxy-ester **5** gave a *monotoluene*-p-*sulphonate*, with TsCl in Py, mp 163°: IR ν_{max} cm^{-1}: 1704, 1655, 1595, 1175, 950, 910, 890; ^{1}H NMR: δ 0.87 (3H, *s*, 20-H), 1.22 (3H, *s*, 18-H), 2.42 (3H, *s*, Ar-Me), 3.62 (3H, *s*, OMe), 4.25 (1H, *dd*, $J = 4$ and 12 Hz, 3-H), 4.75 (2H, 17-H), 7.35 and 7.82 (each 2H, *d*, $J = 8$ Hz, Ar-H).

Hydrolysis of the acetoxy-acid **1**. The acid (20 mg) was treated with 10% aq. ethanolic KOH (2 ml) overnight. The soln was acidified and the hydroxy-acid **3** recovered in EtOAc. It had mp 205–207° (lit. [6] 207–210°); ^{1}H NMR: δ 0.99 (3H, *s*, 20-H), 1.45 (3*H*, *s*, 18-H), 2.65 (1H, *m*), 3.14 (1H, *m*, 3-H), and 4.75 (2H, *m*, 17-H).

Oxidation of the hydroxy-acid **3**. The hydroxy-acid (40 mg) in Me_2CO (5 ml) was treated with excess 8 N CrO_3 at room temp. for 1 hr. MeOH was added and the product recovered in EtOAc, and chromatographed on Si gel to afford the nor-ketone **7** which crystallized from petrol as needles, mp 75°. [Found: M^+ 272.213. Calc. for $C_{19}H_{28}O$: 272.214.] ^{1}H NMR: δ 0.98 (*d*, $J = 7$ Hz), 1.19 (*s*), 1.20 (*d*, $J = 7$ Hz), 4.80 (*m*, 7-H). Irradiation at δ 2.32 collapsed the doublet at δ 0.98 and affected the signal at δ 1.20. MS *m/e* (rel. int.): 272 (29), 257 (52), 229 (99), 213 (58), 211 (69), 131 (51), 105 (64), 91 (100).

Oxidation of the hydroxy-ester **5**. The ester **5** (50 mg) in Me_2CO (5 ml) was treated with the 8 N CrO_3 reagent (0.1 ml) for 10 min at room temp. MeOH was added, the solvent was evapd and the keto-ester recovered in EtOAc. The keto-ester **6** crystallized from petrol as needles, mp 120–125°. [Found: M^+ 330.217. $C_{21}H_{30}O_3$ requires 330.219]. IR ν_{max} cm^{-1}: 1730 (br), 1650, 890; ^{1}H NMR δ 1.13, 1.35 and 3.65 (each 3H, *s*, 20-H, 18-H and OMe), 4.80 (2H, *m*, 17-H); MS *m/e* (rel. int.): 330 (20), 287 (51), 255 (47), 227 (54), 187 (47), 131 (42), 105 (67), 91 (100).

Reduction of the hydroxy-ester **5**. The ester **5** (30 mg) in Et_2O (10 ml) was treated with $LiAlH_4$ (20 mg) for 1 hr. The excess reagent was destroyed with dil HCl and the product recovered in EtOAc to afford the diol **2**, identified by its IR spectrum. Under similar conditions, the acetoxy-ester **4** (25 mg) gave the same diol, identified by its IR spectrum. The diol **4** was identical (^{1}H NMR) to an authentic sample of *ent*-3β,19-dihydroxy-kaur-16-ene, kindly provided by Professor J. MacMillan F.R.S.

Acknowledgements—We thank N.A.T.O. for a research grant and Professor J. MacMillan F.R.S. for kindly sending us an authentic sample of *ent*-3β,19-dihydroxy-kaur-16-ene.

REFERENCES

1. Savona, G., Piozzi, F. and Hanson, J. R. (1978) *Phytochemistry* **17**, 2132.
2. Savona, G., Piozzi, F. and Hanson, J. R. (1978) *J. Chem. Soc. Perkin Trans.* **1**, 1080.
3. Hanson, J. R., Siverns, M., Piozzi, F. and Savona, G. (1976) *J. Chem Soc. Perkin Trans.* **1**, 114.
4. Baddeley, G. V., Jefferies, P. R. and Retallack, R. W. (1964) *Tetrahedron* **20**, 1983.
5. Middleton, E. J. and Jefferies, P. R. (1968) *Aust. J. Chem.* **21**, 2349.
6. Lunnon, M. W., MacMillan, J. and Phinney, B. O. (1977) *J. Chem. Soc. Perkin Trans.* **1**, 2308.
7. Popa, D. P. and Pasechnik, G. S. (1974) *Khim. Prir. Soedin.* **10**, 447.