were recorded on an Jeol-IMS-OLSG-2 spectrometer, ionisation potential 75 eV. CC was performed on Si gel (0.05–0.20 mm). All the new products here reported gave satisfactory elemental analyses.

Plant material. Sideritis sicula was collected on the high summits of Madonie Mounts (Sicily). A specimen is deposited in the Herbarium of the 'Orto Botanico, University-Palermo'.

Extraction of the diterpenes. The inflorescence were ground and extracted (Soxhlet) with petrol for 48 hr. The solvent was removed under red. pres. and the residue chromatographed on a column. The fraction eluted with cyclohexane–Et_2O (9:1) yielded sideripol (**1**) (100 mg); elution with cyclohexane–Et_2O (1:1) gave eubol (300 mg), mp 194–195° (from EtOAc), mp, IR and NMR identical with reported data [6]: mmp did not depress. Elution with Et_2O–EtOAc (3:7) gave epoxysideritriol (15 mg).

Sideripol (**1**). Mp 121–122° (from petrol); positive TNM test: IR: 3448 cm^{-1} (OH), 1709 and 1266 cm^{-1} (AcO), 3030 and 827 cm^{-1} (trisubstituted C=C); MS: 346 (M^+), 328 (M-H_2O), 313 (M-H_2O—Me), 287 (M-OAc), 255 (M-CH_2OAc—H_2O), 109 (ring A, $C_6H_7Me_2$); NMR: δ 0.82 (3H, s, 4α-Me), 1.08 (3H, *s*, 10α-Me), 1.72 (3H, *d*, J = 1.5 Hz, 16-Me), 2.06 (3H, *s*, OAc), 2.30 (1H, *br*, 13-H), 3.43 and 4.03 (2H, q_{AB} 10.5 Hz, 4β-C$\underline{H}_2$OAc), 3.58 (1H, *br*, 7α-H), 5.49 (1H, *br*, $W_{\frac{1}{2}}$ = 4.5 Hz, 15-H).

By alkaline hydrolysis with 5% KOH–EtOH (at room temp. for 24 hr) it yielded sideridiol (**3**) [2], mp 195–196°; by acetylation diacetoxysideridiol, mp 128–129° [2], (IR spectra superimposable).

Partial acetylation of sideridiol (**3**). To a soln of (**3**) (200 mg) in Py (5 ml), cold Ac_2O (2.5 ml) was added at 0° and the mixture left at this temp. for 15 min. Dry CC (cyclohexane–EtOAc, 3:1) of the residue gave some diacetate (3 mg), 18-monoacetate (70 mg), identical (mmp, IR, NMR) with natural sideripol (**1**) and the 7-monoacetate (**4**) (8 mg), identified by comparison with an authentic marker [2] (mmp, TLC, IR, NMR) and starting material (75 mg).

Epoxysideritrol (**2**). $C_{20}H_{32}O_4$, mp 234–235° (from EtOAc); TNM reaction: negative; IR: 3390–3279 cm^{-1} (OH); MS: 305 (M-CH_2OH), 287 (M-CH_2OH—H_2O), 270 (M-CH_2OH—H_2O—OH), 256 (M-2CH_2OH—H_2O), 109 *m/e* (ring A, $C_6H_7Me_3$); NMR (60 MHz, pyridine-d_5) δ0.95 (3H, *s*, 4α-Me), 1.08 (3H, *s*, 10α-Me), 2.70 (1H, *br*, 13α-H), 3.45 and 3.66 (2H, $q_{AB}J$ = 11 Hz, 4β-C$\underline{H}_2$OH), 3.81 (1H, *s*, 15-H), 4.12 (1H, *t* J = 2 Hz, 7α-H), 4.45 and 4.0 (2H, q_{AB} J = 12 Hz, 16β-C$\underline{H}_2$OH).

Triacetylepoxysideritriol (**5**). Obtained by reaction with Py–Ac_2O; mp 138–140° (from cyclohexane); NMR δ0.81 (3H, *s*, 4-Me, 1.06 (3H. *s*, 10—Me), 2.02 (3H, *s*, OAc), 2.07 (6H, *s*, 20Ac), 2.75 1H, *br*, 13α-H), 3.13 (1H, *s*, 15-H), 3.69 (2H, *s*, 4β-C$\underline{H}_2$OAc), 4.08 and 4.63 (2H, q_{AB} J = 12 Hz, 16β-C$\underline{H}_2$OAc), 4.78 (1H, *br* $W_{\frac{1}{2}}$ = 6 Hz, 7α-H). The products (**2**) and (**5**) were also prepared by treatment of sideritriol (**6**) and triacetylsideritriol (**7**) [4] with *p*-nitroperbenzoic acid in ether at room temp. for 24 hr as described for similar derivatives [3, 5, 8].

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REFERENCES

1. Venturella, P. and Bellino, A. (1963) *Atti Accad. Sci. Lettere Arti Palermo* **24**, 195.
2. Piozzi, F., Venturella, P., Bellino, A. and Mondelli, R. (1968) *Tetrahedron* **24**, 4073.
3. Piozzi, F., Venturella, P., Bellino, A. and Mondelli, R. (1968) *Ric. Sci. Riv.* **38**, 462.
4. Piozzi, F., Venturella, P., Bellino, A. and Selva, A. (1969) *Gazz. Chim. Ital.* **99**, 582.
5. Venturella, P., Bellino, A. and Piozzi, F. (1970) *Atti Accad. Sci. Lettere Arti Palermo* **30**, 171.
6. Venturella, P. and Bellino, A. (1977) *Experientia* **33**, 1270.
7. Briggs, L. H., Cambie, R. C. and Rutledge, P. S. (1963) *J. Chem. Soc.* 5374.
8. Venturella, P., Bellino, A. and Piozzi, F. (1975) *Phytochemistry* **14**, 1451.

Phytochemistry, 1978, Vol. 17, pp. 812–814 Pergamon Press. Printed in England

THE MICROBIOLOGICAL TRANSFORMATION OF EPICANDICANDIOL, *ENT*-7α,18-DIHYDROXYKAUR-16-ENE, BY *GIBBERELLA FUJIKUROI*

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Key Word Index—*Gibberella fujikuroi*; microbiological transformation; kauranoid diterpenes.

Abstract—Incubation of *ent*-7α,18-dihydroxykaur-16-ene with *Gibberella fujikuroi* affords *ent*-7α,18,19-trihydroxykaur-16-ene and *ent*-7α,18-dihydroxykaur-16-en-19-oic acid. There was no transformation into 7,18-dihydroxykaurenolide.

INTRODUCTION

The microbiological transformation of artificial substrates by fungi can be divided into two groups. One which is typified by the hydroxylation of steroids utilizes induced enzyme systems with a definite regiospecificity but of low substrate specificity whilst the other, of which there are relatively few examples, utilizes the natural biosynthetic pathway and substrates related to the normal metabolites. Thus steviol (**1**) which is related to the normal metabolite of *Gibberella fujikuroi*, *ent*-kaur-16-en-19-oic acid, is metabolized [1] to 7,13-dihydroxykaurenolide (**2**), a 13-hydroxy analogue of the normal metabolite This work has subsequently been extended in an elegant

manner by MacMillan [2a, 2b] to the partial synthesis of 13-hydroxylated gibberellins. 7,18-Dihydroxykaurenolide (3) is the major kaurenolide metabolite of *Gibberella fujikuroi* [3]. It is formed via *ent*-kaur-16-en-19-oic acid, 7β-hydroxylation, conversion to 7β-hydroxykaurenolide and finally 18-hydroxylation [4]. 7α,18-Dihydroxykaur-16-ene, epicandicandiol (4), isolated from *Sideritis candicans* [5, 6], contains some stages of this biosynthesis (e.g. 18-hydroxylation) but lacks the 19 → 6 lactone ring. Its microbiological transformation by *Gibberella fujikuroi* was therefore of interest in the context of the substrate-specificity of the C-19 and C-6 oxidative sequence.

RESULTS

Incubation of epicandicandiol (4) with *Gibberella fujikuroi* ACC 917 for 4 days gave a triol, $C_{20}H_{32}O_3$ (5). The PMR spectrum showed only one $-\overset{|}{\underset{|}{C}}-Me$ resonance and an additional $-\overset{|}{\underset{|}{C}}-CH_2OH$ signal. The compound readily formed a triacetate. The location of the additional hydroxyl group at C-19 rather than C-20 followed from the ready formation of an 18,19-acetonide with acetone and copper sulphate. An acetonide cannot be formed between C-18 and C-20. As would be expected the H-20 signal showed a significant deshielding (Δδ0.2 ppm in Py) between epicandicandiol and the triol.

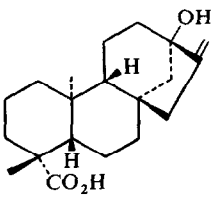
An acidic metabolite was detected when the incubation was carried out in the presence of AMO 1618 which inhibits [7] the endogenous formation of kauranoid and hence gibbane metabolites, by *G. fujikuroi*. The acid (6), which was isolated as its methyl ester (7), was related to the triol (5) by reduction of the methyl ester with lithium aluminium hydride. The carboxyl group was assigned to the 19-position since the 20-H signal in the NMR spectrum of the methyl ester were co-incident with those of methyl ent-7α-hydroxykaur-16-en-19-oate (δ 0.87 v 0.86 ppm) and did not show the downfield shift which would be associated with a diaxial C-19 CH_2OH interaction. The methyl ester had an R_f value comparable to that of methyl gibberellate on TLC and this obscured it in the non-inhibited fermentation.

Epicandicandiol (4) was tritiated at C-18 as follows. Careful hydrolysis of the 7,18-diacetate afforded the 7-monoacetate which was oxidized to the aldehyde (8) [8]. This was reduced, first with sodium ^{3}H-borohydride and then with lithium aluminium hydride to afford [18-^{3}H]-epicandicandiol (4). Incubation with *Gibberella fujikuroi*, using an inhibited culture, gave the triol (5) (16.5% incorporation) and the acid (6) (isolated as its methyl ester, 14.8% incorporation). The retention of tritium by the methyl ester (7) is further proof that the carboxyl group is located at C-19 rather than C-18. The neutral fraction was assayed by dilution analysis for radioactive 7,18-dihydroxykaurenolide (3). However this was not labelled. No TLC evidence could be found for additional acidic and possibly gibbane metabolites.

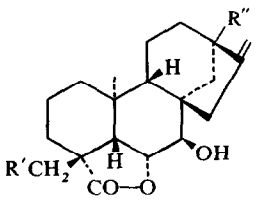
It is interesting to note that although hydroxylation and oxidation at C-19 occurs, the lactonization and ring contraction stages, involving reaction at C-6, are inhibited by the additional hydroxyl group at C-18. The further oxidation at C-6 in the kaurenolide series leading to the anhydride, fujenal, also does not appear to happen with 7,18-dihydroxykaurenolide [9]. 3-Hydroxylation has also been shown [2] to be sensitive to substitution elsewhere in the molecule.

EXPERIMENTAL

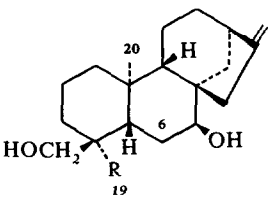
Incubations of epicandicandiol (4) *with* G. fujikuroi. (i) *G. fujikuroi* (ACC 917) was grown as previously described [10] in shake culture (100 ml medium per flask) for 4 days. Epicandicandiol (4) (380 mg) in EtOH (95 ml) was distributed between 95 flasks. After a further 3 days growth, the broth was filtered, adjusted to pH 2 with HCl and extracted with EtOAc. The extract was separated into acidic and neutral fractions with $NaHCO_3$. The neutral fraction (670 mg) was chromatographed on Si gel (dry column). Elution with EtOAc–petrol (1:1) gave *ent-7α,18,19-trihydroxykaur-16-ene* (65 mg) which crystallized from EtOAc as prisms, mp 193–197°, $[\alpha]_D$ −35° (MeOH *c* 0.15). (Found: C, 70.4; H, 9.9. $C_{20}H_{32}O_3.H_2O$ requires C, 71.0; H, 10.1%); IR ν_{max} cm^{-1}: 3350, 3090, 1650, 1080, 1040, 1020, 880; PMR (Py d_5): δ 1.10 (3H, *s*, 20-H_3), 3.7 (1H. *br s*, 7-H), 3.87, 3.95, 4.16 and 4.18 (each 1H, *d*, *J* = 12 Hz, H-18 and H-19), 4.87 (2H, *br s*, 17-H_2); MS *m/e*: 320, 302, 284, 272, 271, 254, 253, 241, 239. The triacetate, prepared with Ac_2O in Py, was a gum, PMR ($CDCl_3$): δ



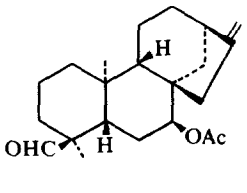
(1)



(2) R′ = H, R″ = OH
(3) R′ = OH, R″ = H



(4) R = Me
(5) R = CH_2OH
(6) R = CO_2H
(7) R = CO_2Me



(8)

1.09 (3H, *s*) 2.01 (9H, *s*, 3-OAc), 3.77 and 3.93 (each 1H, *d*, *J* = 12 Hz), 3.98 and 4.24 (each 1H, *d*, *J* = 12 Hz), 4.75 (3H, *br s*, =CH_2,C<u>H</u>.OAc). (ii) *G. fujikuroi* was grown as above but AMO 1618 (3.54 mg) in EtOH (0.1 ml) was added to each flask at the time of inoculation. After 4 days growth, epicandicandiol (84 mg) in EtOH (25 ml) was distributed between 25 flasks. The flasks were harvested as above after a further 3 days growth ent-7α,-18,19-Trihydroxykaur-16-ene (15 mg) was obtained from the neutral fraction. The acid fraction was methylated with diazomethane and chromatographed on Si gel to afford *methyl ent-7α,-18-dihydroxykaur-16-en-19-oate* (9 mg) which crystallized from EtOAc as prisms, mp 228–230° (Found: MS 330.2210 M^+-H_2O, $C_{21}H_{30}O_3$ requires 330.2194), PMR ($CDCl_3$): δ 0.87 (3H, *s*) 3.61 (2H, *s*, CH_2OH), 3.63 (3H, *s*. OMe). 3.65 (1H, *br s*, C<u>H</u>.OH), 4.77 (2H, *br s*, =CH_2). (iii) Epicandicandiol-[18-^{3}H] (6 mg, 1.0926 mCi) was distributed between two flasks of *G. fujikuroi* which had been grown as in (ii). After a further 3 days growth, the fermentation was harvested as before. The neutral fraction afforded ent-7α,18,19-trihydroxykaur-16-ene (0.179 mCi, 16.5%). The fraction was subjected to TLC against authentic 7,18-dihydroxykaurenolide. The region corresponding to the kaurenolide was eluted from the plate and diluted with authentic 7,18-dihydroxykaurenolide (10 mg). After 3 recrystallizations it was inactive. The acid fraction was methylated with CH_2N_2 and gave, after PLC, methyl ent-7α,18-dihydroxykaur-16-en-19-oate (0.163 mCi, 14.8%).

Acetonide of ent-7α,18,19-trihydroxykaur-16-ene. The triol (40 mg) in Me_2CO (3 ml) was treated with $CuSO_4$ (240 mg) at room temp. for 5 hr. The soln was filtered, the solvent was evapd and the residue chromatographed on Si gel in EtOAc–petrol (3:7) to afford the acetonide as a gum. (Found: 360.2647, $C_{23}H_{36}O_3$ requires 360.2664; 345.2429, M-15, $C_{22}H_{33}O_3$ requires 345.2429). PMR ($CDCl_3$): δ 0.93 (3H, *s*, 20-H_3) 1.30 and 1.35 (each 3H, *s*, C.Me_2), 3.21 and 5.54 (each 1H, *d*, J = 12 Hz, CH_2O) 3.60 (3H, *br s*, CH_2O and CH.OH), 4.77 (2H, *br s*, $=CH_2$); MS *m/e*: 360, 345 (100%), 342, 327, 272, 257, 254, 239, 226, 211.

Reduction of methyl ent-7,18-dihydroxykaur-16-en-19-oate. The methyl ester (6 mg) in dry Et_2O (3 ml) was added to a suspension of LiAlH (25 mg) in the same solvent (5 ml). After 6 hr H_2O and dil.HCl were carefully added and the product was recovered in Et_2O to afford the triol (3 mg) which was identified by TLC and its PMR spectrum.

Epicandicandiol-[18-^3H]. *ent*-7α-Acetoxykaur-16-en-18-al (20 mg) [8] was added to a soln of NaB^3H_4 (1.5 mg, 10 mCi) in MeOH (3 ml). After 2 hr the MeOH was evapd, Et_2O (5 ml) was added followed by excess LiAlH. After a further 3 hr H_2O and dil.HCl were added and the product was recovered in Et_2O, to afford epicandicandiol-[18-^{3}H] (18 mg, 3.0878 mCi).

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REFERENCES

1. Hanson, J. R. and White, A. F. (1968) *Tetrahedron* **24**, 6291.
2. (a) Bearder, J. R., MacMillan, J., Wels, C. M. and Phinney, B. O. (1975) *Phytochemistry* **14**, 1741;
2. (b) Bearder, J. R., Frydman, V. M., Gaskin, P., MacMillan, J., Wels, C. M. and Phinney, B. O. (1976) *J. Chem. Soc. Perkin* 1 173.
3. Cross, B. E., Galt, R. H. B. and Hanson, J. R. (1963) *J. Chem. Soc.* 3783.
4. Hanson, J. R. (1971) in *Progress in the Chemistry of Organic Natural Products* (Herz, W., Grisebach, H. and Kirby, G. W. eds) Vol. 29, p. 395. Springer-Verlag, Vienna.
5. Breton, J. L., Gonzales, A. G., Rocha, J. M., Panizo, F. M., Rodriguez, B. and Valverde, S. (1969) *Tetrahedron Letters* 599.
6. Piozzi, F., Venturella, P., Bellino, A., Paternostro, M. P., Rodriguez, B. and Valverde, S. (1971) *Chem. Ind. (Lond.)* 962.
7. Dennis, D. T., Upper, C. D. and West, C. A. (1965) *Plant Physiol.* **40**, 948.
8. Gonzalez, A. G., Fraga, B. M., Hernandez, M. E. and Luis, J. G. (1973) *Tetrahedron* **29**, 561.
9. Hanson, J. R. and Sarah, F. unpublished results.
10. Hanson, J. R., Hawker, J. and White, A. F. (1972) *J. Chem. Soc. Perkin* 1 1892.

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KAURENIC ACID DERIVATIVES FROM *ADENOSTEMMA CAFFRUM*

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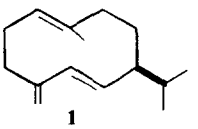
Key Word Index—*Adenostemma caffrum;* Eupatorieae; Compositae ikaurenic acid derivatives; diterpenoids.

All parts of the South African species *Adenostemma caffrum* DC, contain, besides Germacrene D (**1**) the three kaurenic acid derivatives **2**, **3** and **4** identical with those previously isolated from *Eupatorium album* [1] The only previous investigation of a *Adenstemma* species is a report on isolation of the widespread pentaynene [2]. The co-occurrence of the same diterpenes in a *Eupatorium* änd a *Adenostemma* species indicates a close relationship between these two genera.



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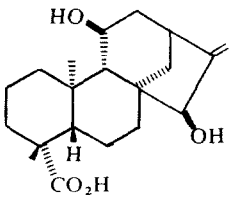

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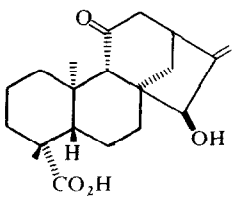

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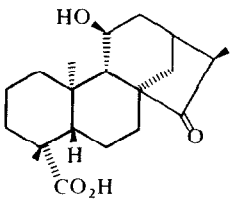

4

EXPERIMENTAL

The air dried plant material (collected in Natal, voucher 77/86) was extracted with Et_2O–petrol (1:2) and the extracts were separated by column chromatography and further by TLC (Si gel GF 254) using Et_2O–petrol mixtures as solvents, 144 g of roots afford 20 mg **1**, 40 mg **2**, 5 mg **4** and 10 mg **3**, while 285 g aerial parts yielded 30 mg **1**, 40 mg **2**, 22 mg **4** and 30 mg **3**. The structures were elucidated by 270 MHz-^{1}H-NMR and by transformation of the acids to methyl esters and by

* Part 130 in the series 'Naturally Occurring Terpene Derivatives'; for part 129 see: Bohlmann, F. and Zdero, C. (1978) *Phytochemistry* **17**, 565.