Preparation of Kaurenolide and its 5α,6α(H)-Isomer

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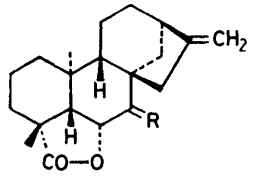
In the preparation of kaurenolide, essentially by the literature method, the formation of *ent*-6-oxokaur-16-en-19-oic acid from the toluene-*p*-sulphonate of 7β-hydroxykaurenolide was found to be dependent on the quality of the lithium iodide monohydrate used. *ent*-6-Hydroxykaura-6,16-dien-19-oic acid 19,6-lactone, prepared from the toluene-*p*-sulphonate of 7β-hydroxykaurenolide, zinc dust, and sodium iodide in refluxing 2-methoxyethanol, was found to be hydrolysed by base to *ent*-6-oxo-5β-kaur-16-en-19-oic acid, which gave the 5α,6α(H) isomer of kaurenolide upon reduction.

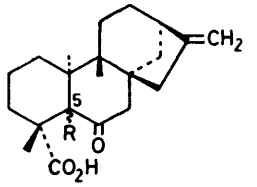
KAURENOLIDE [*ent*-6β-hydroxykaur-16-en-19-oic acid 19,6-lactone (1)] was required for biosynthetic studies.[1] Cross *et al.*[2] have described the preparation of *ent*-6-oxokaur-16-en-19-oic acid (7) by treatment of the toluene-*p*-sulphonate (3) of the fungal metabolite, 7β-hydroxykaurenolide (2), with dry lithium iodide in refluxing collidine. The conversion of the 6-oxo-acid (7) into kaurenolide (1) has been described by Hanson.[3] Re-investigation of this route led to complications which are now reported.

Treatment of the toluene-*p*-sulphonate (3) with an old batch of lithium iodide monohydrate which had been dried at 100 °C overnight under vacuum gave a very low yield of the 6-oxo-acid (7); the main product was the enol lactone (11). In the original paper describing their reaction, Cross *et al.*[2] did not discuss the mechanism but did report that treatment of the 16,17-dihydro-derivative of the toluene-*p*-sulphonate (3) with collidine in the absence of lithium iodide gave the 16,17-dihydro-derivative of the enol lactone (11). Since the enol lactone (11) is a likely intermediate in the formation of the 6-oxo-acid (9) from the toluene-*p*-sulphonate (3), the use of undried sodium iodide, and of collidine to which water had been added, were tried. However, the yield of the 6-oxo-acid (9) was not improved from the 7β-toluene-*p*-sulphonate (3), its 7α-epimer (4), or the 7β-methanesulphonate (5). Replacing lithium iodide by sodium iodide, lithium bromide, or a mixture of the latter two salts gave mixtures of the enol lactone (11) and the rearranged lactone (12). Reaction of 7β-hydroxykaurenolide (2) with phosphorus pentachloride, as described by Hanson,[3] gave the enol lactone (11), the rearranged lactone (12), and the 6-oxo-acid (7) in variable yield but the yield of the 6-oxo-acid (7) did not exceed 30%. With phosphoryl chloride in pyridine, 7β-hydroxykaurenolide (2) gave mainly the enol lactone (11), with minor amounts of the rearranged product (12). However the enol lactone (11) was best prepared from the toluene-*p*-sulphonate (3), sodium iodide, and zinc dust in refluxing methoxyethanol.

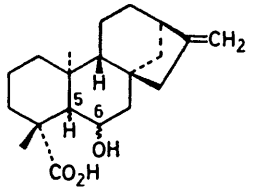
Eventually the practical problem of preparing the 6-oxo-acid (7) from 7β-*p*-tolylsulphonyloxykaurenolide (3) in good yield was solved by using lithium iodide monohydrate from a newly opened bottle. The enol lactone (11) was similarly converted into the 6-oxo-acid (7). However the yields decreased with the age of the lithium iodide monohydrate, kept on the shelf, although there was no apparent decomposition of the reagent. The efficacy of old lithium iodide monohydrate could not be restored by heating at 100 °C. These observations were confirmed by repeated experiments and we have no explanation to offer.



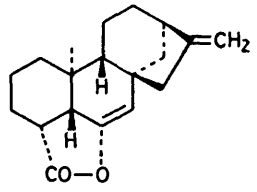
(1) R = H_2
(2) R = H, β-OH
(3) R = H, β-$OSO_2C_6H_4Me$-*p*
(4) R = H, α-$OSO_2C_6H_4Me$-*p*
(5) R = H, β-OSO_2Me
(6) R = H, β-OCSPh



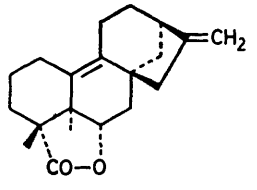
(7) R = 5β-H
(8) R = 5α-H



(9) 5β-H, 6α-OH
(10) 5α-H, 6β-OH



(11)

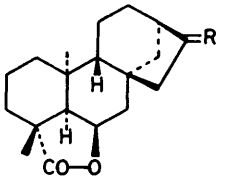


(12)

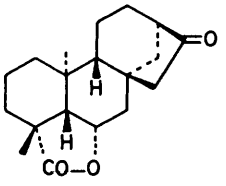
Kaurenolide (1) was prepared from the 6-oxo-acid (7) by reduction with sodium borohydride to the 6α-alcohol (9), which was lactonised using dicyclohexylcarbodiimide. It was identical with the kaurenolide (1) prepared [1] by reduction of the thiobenzoate (6) with tri-n-

butylstannane. Both methods gave kaurenolide (1) with a higher m.p. than previously reported.[3]

Alkaline hydrolysis of the enol lactone (11) did not provide an alternative preparation of the 6-oxo-acid (7). Only a low yield of the 6-oxo-acid was obtained. The main product was an isomer which was also obtained from the 6-oxo-acid (7) by similar treatment with alkali and is assigned the 5α-structure (8). This 5α-epimer (8) was reduced to an alcohol which was rapidly lactonised at room temperature to an isomer of kaurenolide (1). The structures (10) and (13) for the alcohol and the isomeric kaurenolide are assigned for the following reasons.



(13) R = CH_2
(14) R = O



(15)

Reduction of the epimeric 6-oxo-acids (7) and (8) with sodium borohydride in tetrahydrofuran and ethanol occurred at comparable rates but, in tetrahydrofuran–methanol the 5α-epimer (8) was reduced much more slowly. From molecular models, the 6-position is more sterically hindered in the 5α-epimer (8) than in the 5β-epimer (7) and the α-face of the 5α-epimer (8) is more accessible than the β-face. This and the spontaneous lactonisation of the alcohol obtained from the 5α-epimer leads to the structures (10) and (13).

In the n.m.r. spectrum of the isomeric kaurenolide (13), the 6-proton signal occurred as a double triplet at δ 4.67 with measured J values of 8, 8, and 11 Hz. The 6-proton signal of kaurenolide (1) was obscured by the signals of the exocyclic methylene protons. Both kaurenolide (1) and the isomeric kaurenolide (13) were therefore oxidised to their corresponding norketones (15) and (14). In the n.m.r. spectrum of kaurenolide norketone (15) the 6-proton signal occurred at δ 4.86 as a double triplet with measured J values of 6, 8, and 8 Hz. The 6-proton signal of the isomeric norketone (14) occurred at δ 4.76 as a double triplet with J values of 8, 8, and 11 Hz. These J values are consistent with a chair–chair conformation for the A/B ring system in both lactones.

EXPERIMENTAL

For general experimental details see ref. 4.

ent-6-*Oxokaur*-16-*en*-19-*oic Acid* (7).—(*a*) Following the procedure of Cross *et al.*,[2] *ent*-6β-hydroxy-7-*p*-tolylsulphonyloxykaur-16-en-19-oic acid 19,6-lactone (3) (1.31 g; m.p. 170—172°; lit.,[2] 167—169°) in collidine (35 ml) was refluxed for 5 h with lithium iodide monohydrate (1.5 g; newly opened bottle from B.D.H. Ltd.). The usual work-up gave a semi-crystalline product (760 mg) which was chromatographed on a silica gel column, developed with light petroleum–ethyl acetate, to give the required 6-oxo-acid (7), crystallising from ethyl acetate in needles (340 mg) m.p. 255—256° (lit.,[2] 205—206°; lit.,[3] 263—265°) (Found: C, 75.6; H, 9.3. Calc. for $C_{20}H_{28}O_3$: C, 75.9; H, 8.9%); δ 1.0 (s, 3 H), 1.24 (s, 3 H), and 4.84 (br, 2 H); m/z 316 (M^+, 19%), 272 (55), 257 (100), 137 (64), and 109 (79).

(*b*) The enol lactone (11) (1.48 g) and lithium iodide monohydrate (2.5 g; fresh from B.D.H. Ltd.) were refluxed in collidine (30 ml) for 3.5 h. The usual work-up and purification of the product (1.46 g) as in (*a*) gave the oxo-acid (7) (630 mg).

ent-6-*Hydroxykaura*-6,16-*diene*-19-oic *Acid* 19,6-*Lactone* (11).—(*a*) 7β-Hydroxykaurenolide (2) (400 mg) and phosphoryl chloride (800 μl) in pyridine (12 ml) were refluxed for 1.5 h. The mixture was poured into water, the pH was lowered to below 2.0, and the products (400 mg) were recovered in ethyl acetate. Chromatography of the product mixture on a silica gel column with light petroleum gave the rearranged lactone (12), which crystallised from ethyl acetate–light petroleum in needles (24 mg), m.p. 244—245° (lit.,[3] 233—234°), and was identified from its n.m.r. spectrum. Further elution gave the enol lactone (11), which crystallised from acetone–light petroleum in plates (155 mg), m.p. 211—212° (lit.,[3] 202—204°), identified from its i.r. spectrum; m/z 298 (M^+, 67%), 270 (15), 255 (35), 227 (25), 199 (37), and 109 (100).

(*b*) *ent*-6β-Hydroxy-7α-*p*-tolylsulphonyloxykaur-16-en-19-oic acid 19,6-lactone (3) (90 mg) was refluxed for 2 h with sodium iodide (150 mg) and zinc powder (130 mg) in 1,2-dimethoxyethane (2 ml). Dilution with water, extraction with ethyl acetate, and p.l.c. gave the enol lactone (11) (50 mg).

ent-6β-*Hydroxykaur*-16-*en*-19-*oic Acid* (9).—The 6-oxo-acid (7) (570 mg), in tetrahydrofuran (50 ml) and ethanol (50 ml) was treated with sodium borohydride (250 mg) for 2 h at room temperature. Work-up in the usual way gave crystalline material (566 mg) which was recrystallised from acetone to give the *hydroxy-acid* (9) as needles, m.p. 250—255° (sublimation) (Found: M^+, 318.218. $C_{20}H_{30}O_3$ requires M, 318.219); m/z 318 (M^+, 8%), 300 (42), 285 (62), 241 (57), 211 (68), and 109 (100).

ent-6β-*Hydroxykaur*-16-*en*-19-*oic Acid* 19,6-*Lactone* (*Kaurenolide*) (1).—The hydroxy-acid (9) (400 mg) and dicyclohexylcarbodi-imide (400 mg) in pyridine (6 ml) were heated for 4 h on a steam-bath, then cooled. The crystalline dicyclohexylurea was removed by filtration, and the filtrate was poured into water and extracted below pH 2 with ethyl acetate. Recovery from the ethyl acetate gave a crystalline solid (361 mg), which was recrystallised from acetone to give kaurenolide (1) as prisms, m.p. 234° (sublimation at 220°) (lit.,[3] 204—205°), identical with the compound prepared by Beale *et al.*[1]

ent-6β-*Hydroxy*-16-*oxo*-17-*norkauran*-19-*oic Acid* 19,6-*Lactone* (15).—Kaurenolide (1) (100 mg) and osmium tetraoxide (3 mg) in tetrahydrofuran (5 ml) and water (5 ml) were stirred at 0 °C for 10 min. Sodium periodate (150 mg) was added and the mixture was allowed to warm to room temperature; stirring was continued for 24 h. The tetrahydrofuran was removed and the aqueous residue, after dilution with water, was extracted with ethyl acetate. P.l.c. of the crude product gave the norketone (15), which crystallised from acetone–light petroleum as needles (31 mg), m.p. 268—296° (lit.,[3] 264—265°); δ 0.91 (s, 3 H), 1.28 (s, 3 H), and 4.9 (dt, J 6, 8, and 8 Hz, 1 H); m/z 302 (M^+, 22%), 258 (44), 243 (100), 143 (31), 109 (34), 99 (35), 93 (33), and 91 (40).

Alkaline Hydrolysis of ent-6-*Hydroxykaura*-6,16-*dien*-19-*oic Acid* 19,6-*Lactone* (11).—A solution of the enol lactone (11) (600 mg) in t-butyl alcohol (30 ml) and M-potassium hydroxide (30 ml) was refluxed for 2 h. The alcohol was removed and, the residue was diluted with water and extracted with ethyl acetate; the extract was discarded. The aqueous layer was adjusted to below pH 2.0 and extracted with ethyl acetate to give an acidic fraction, which was chromatographed on a silica gel column. Elution with light petroleum containing 10% ethyl acetate gave ent-6-*oxo*-5β-*kaur*-16-*en*-19-*oic acid* (8) (262 mg) admixed with *ca.* 10% *ent*-6-oxokaur-16-en-19-oic acid (7). Recrystallisation of the mixture from ethyl acetate–light petroleum gave the pure *acid* (8) as prisms, m.p. 257—259° (decomp.) (Found: C, 75.7; H, 8.9. $C_{20}H_{28}O_3$ requires 75.9; H, 8.9%); δ 1.20 (s, 3 H), 1.32 (s, 3 H), 3.56 (s, 1 H), 4.76 (br, 1 H), and 4.83 (br, 1 H); *m/z* 316 (M^+, 57%). 298 (36), 270 (28), 203 (40), 119 (30), 109 (100), and 97 (37); $\nu_{max.}$ 2 940, 1 720, 1 370, 1 180, and 895 cm^{-1}.

ent-6α-*Hydroxy*-5β-*kaur*-16-*en*-19-*oic Acid* 19,6-*Lactone* (13).—The 6-oxo-acid (8) (150 mg) in tetrahydrofuran (10 ml) and ethanol (10 ml) was reduced at room temperature for 2 h with sodium borohydride (50 mg). Work-up as usual followed by p.l.c. of the crude product gave the hydroxy-acid (10) (105 mg); δ 1.42 (s, 3 H), 1.44 (s, 3 H), 4.80 (br, 2 H), and 4.36 (m, *J* 6, 6, and 11 Hz, 1 H). This acid was spontaneously lactonised over several days to give in quantitative yield, the *kaurenolide* (13), which crystallised from acetone–light petroleum in prisms, m.p. 125—128° (Found: C, 79.9; H, 9.5. $C_{20}H_{28}O_2$ requires C, 80.0; H, 9.4%); δ 1.16 (s, 3 H), 1.48 (s, 3 H), 4.5—4.9 (m, 1 H), and 4.84 (br, 2 H); *m/z* 300 (M^+, 10%), 241 (43), 173 (39), 123 (32), 119 (77), 109 (65), 105 (44), and 91 (100); $\nu_{max.}$ 2 940, 2 880sh, 1 768, and 1 723 cm^{-1}.

ent-6α-*Hydroxy*-16-*oxo*-17-*nor*-5β-*kauran*-19-*oic Acid* 19,6-*Lactone* (14).—The lactone (13) (133 mg) in tetrahydrofuran (7.5 ml) and water (7.5 μl) was oxidised with osmium tetraoxide (2—3 mg) and sodium periodate (150 mg) as for kaurenolide (1). The product, purified by p.l.c. then crystallisation from acetone–light petroleum, gave the *norketone* (14) as rods (40 mg), m.p. 204—206° (Found: M^+, 302.188. $C_{19}H_{26}O_3$ requires *M*, 302.188); δ 1.16 (s, 3 H), 1.50 (s, 3 H), 4.76 (dt, *J* 8, 8, and 11 Hz, 1 H); $\nu_{max.}$ 2 958, 1 770, and 1 745 cm^{-1}; *m/z* 302 (M^+, 31%), 258 (100), 243 (93), 215 (65), 109 (46), and 91 (53).

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