On the Structures of the Diterpenes Licamichauxiioic Acids A and B

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The structures of the diterpenes licamichauxiioic acids A and B, isolated from *Licania michauxii*, which had been reported as 15-oxo-*ent*-kaur-9(11),16-dien-19-oic acid (**1**) and 15-oxo-*ent*-kaur-13,16-dien-19-oic acid (**3**), respectively, are not correct. Starting from grandiflorenic acid (**6**) we had prepared a compound with the proposed structure for licamichauxiioic acid A, and its spectroscopic data are different from those given for this acid. In the case of licamichauxiioic acid B, its NMR data are not in accordance with the proposed structure **3**, which also violates Bredt's rule. In addition, we described a useful method for the separation of grandiflorenic and grandiflorolic acids.

We have been interested for the past years in the isolation, chemistry, and microbiological transformation of *ent*-labdane, *ent*-kaurene, and *ent*-trachylobane diterpenes.[1−3] These studies have also permitted the assignment of new corrected structures to *ent*-kaurene derivatives isolated from *Sideritis athoa*[4] and *Aristolochia anguicida*.[5]

Badisa and co-workers have shown that root extracts of *Licania maichauxii* (Chrysobalanaceae) are cytotoxic to cultured human hepatoma and colon carcinoma.[6] Later, following a bioassay-guided fractionation of a root extract of this plant, this group described the isolation of two new diterpenes, licamichauxiioic acids A and B, determining their structures as 15-oxo-*ent*-kaur-9(11),16-dien-19-oic acid (**1**) and 15-oxo-*ent*-kaur-13,16-dien-19-oic acid (**3**), respectively.[7] Recently, pure licamichauxiioic acid B was shown to have 1.5−3 times greater cytotoxic activity than the crude extract of the plant against several cancer cell lines.[8] We now demonstrate that the structures **1** and **3** given to these acids are erroneous.

The spectroscopic data of licamichauxiioic acid A are not in accordance with structure **1**. Thus, the above authors indicated that in its ^{13}C NMR spectrum (Table 1) the resonance at δ 61.8 was due to C-8, by comparison with the *ent*-kaurene derivative **4**,[9] stating "which is ca. 9 ppm downfield from the shift of the same carbon in compound **4**", and ascribed this shift to the presence of the 9,11-double bond. However, a more accurate NMR comparison is that of licamichauxiioic acid A with the diterpene **5**, isolated from a *Jungermannia* species, which possesses the same B, C, and D rings.[10] In compound **5**, C-8 resonates at δ 50.0, while in licamichauxiioic acid A it appears at δ 61.8 (Table 1). These differences also occur with C-7 and C-14, with resonances at δ 32.1 and 34.8 in **1** and δ 24.3 and 39.7 in **5**, respectively. These facts indicated that the structure **1** assigned to licamichauxiioic acid A cannot be correct.

To confirm this assumption, we decided to prepare the acid **1** and the methyl ester **2**, starting from grandiflorenic acid (**6**). We had a mixture of this acid and *ent*-kaur-16-en-19-oic acid (**10**), in a 2:1 ratio, which had been obtained from *Stevia lucida*.[11] It has been stated that the only effective method of separating these two acids was by fractional crystallization, but that they cocrystallized when the ratio dropped to ca. 1:1.[12] We prepared the methyl esters of the mixture of acids, by treatment with diazomethane, but were unable to obtain a pure sample of the grandiflorenic acid methyl ester (**7**). Consequently, we oxidized the mixture of **7** and **11** with selenium dioxide, affording the alylic alcohols **8** and **12**, respectively, which could be separated by chromatography on Si gel eluting with petroleum ether−EtOAc (10%). Compounds **8** and **12** are the methyl esters of 9,11-dehydrograndiflorolic acid (**9**) and grandiflorolic acid (**13**), respectively. The latter acid had been isolated from *Espeletia grandiflora*.[13]

Table 1. ^{13}C NMR (125 MHz) Data

position	**1**[a]	**1**[b]	**2**[a]	**2**[b]	**8**[a]	**9**[a]	**L-A**[c]	**L-B**[d]
1	41.4	41.3	41.5	41.7	40.3	41.0	37.9	39.0
2	20.2	20.3	20.2	20.7	20.2	20.1	19.9	20.1
3	37.5	37.5	37.7	38.1	38.4	38.2	22.3	33.1
4	44.3	44.4	44.4	44.7	44.9	44.7	44.3	44.6
5	47.0	47.4	47.1	47.3	47.4	47.3	45.5	49.5
6	18.4	18.7	18.6	19.2	18.2	18.1	25.1	23.6
7	25.6	25.7	25.7	26.1	23.4	23.3	32.1	32.8
8	49.5	49.4	49.5	49.6	46.5	46.5	61.9	61.7
9	149.6	149.9	149.7	150.4	154.7	154.6	134.9	35.7
10	39.6	39.6	39.5	39.8	40.7	38.8	37.8	38.9
11	120.6	120.1	120.7	120.3	117.1	117.2	123.3	33.9
12	36.2	36.1	36.2	36.3	37.3	37.3	27.8	36.4
13	36.3	36.3	36.3	36.5	39.6	39.5	36.7	136.4
14	40.1	40.8	40.2	40.3	41.1	40.3	34.8	123.4
15	203.2	201.5	203.1	201.7	79.7	79.7	205.9	196.7
16	151.4	151.6	151.4	151.8	163.0	162.8	150.9	154.0
17	115.9	115.2	115.9	115.4	110.2	110.4	115.4	116.0
18	28.4	28.2	28.3	28.4	28.1	28.2	29.2	30.0
19	183.4	184.4	178.2	177.5	177.9	184.1	185.2	181.0
20	22.9	22.8	22.6	23.0	24.0	24.1	17.2	18.8

[a] Solvent: $CDCl_3$. [b] Solvent: C_6D_6. [c] L-A = licamichauxiioic acid A, data from ref 7. [d] L-B = licamichauxiioic acid B, data from ref 7.

The MS of **8** with a molecular ion at *m*/*z* 330.2192 ($C_{21}H_{30}O_3$) showed that only one carbon, C-15 (δ 79.7), had been hydroxylated. In the ^{1}H NMR spectrum the oxymethine proton appears at δ 4.11 (s). The structure of **8** was confirmed via its 2D NMR spectra. Thus, in the HMBC experiment correlations were observed between H-17 and C-15 and between H-15 and C-17.

Swern oxidation[14,15] of **8** gave compound **2**. Its MS and NMR spectra were in accordance with this structure. In the ^{13}C NMR spectrum the 15-oxo carbon resonated at δ 203.1, while the two olefinic C-17 protons appeared in the ^{1}H NMR spectrum at δ 5.43 and 5.90. The HMBC experiment showed correlations between the C-17 protons and C-15. The ^{1}H NMR data of **2** in C_6D_6 were different from those reported for the methyl ester of licamichauxiioic acid A (Table 1). Thus, the structure of this acid needed to be revised.

To confirm this, we also prepared a compound with the structure assigned to licamichauxiioic acid A, starting from pure grandiflorenic acid (**6**). The separation of grandiflorenic acid (**6**) and *ent*-kaur-16-en-19-oic acid (**10**) was achieved by chromatography of

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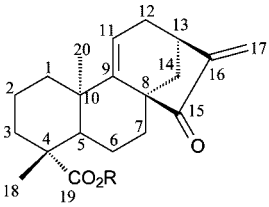

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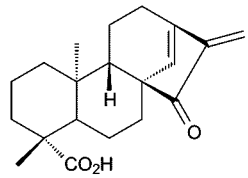

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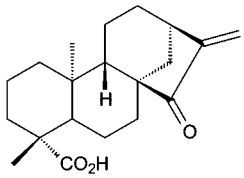

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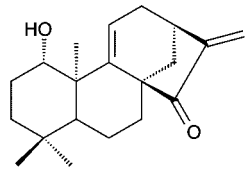

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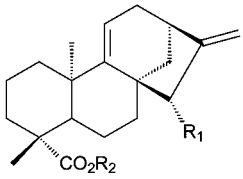
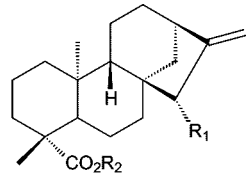
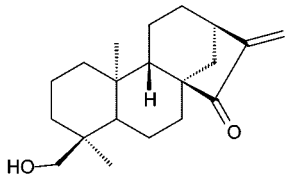


6 $R_1 = R_2 = H$
7 $R_1 = H$ $R_2 = Me$
8 $R_1 = OH$ $R_2 = Me$
9 $R_1 = OH$ $R_2 = H$



10 $R_1 = R_2 = H$
11 $R_1 = H$ $R_2 = Me$
12 $R_1 = OH$ $R_2 = Me$
13 $R_1 = OH$ $R_2 = H$



14

the mixture on Si gel impregnated with $AgNO_3$ (5%), using toluene−EtOAc (3%) as eluent. A 2D-NMR study confirmed the previous assignment of the ^1H and ^{13}C NMR spectra of grandiflorenic acid (**6**).[16]

Treatment of **6** with SeO_2 gave the allylic alcohol **9**. This product had been isolated from *Coespeletia lutescens*[17] and identified as its methyl ester **8**. The spectroscopic data of acid **9** are now given for the first time. Oxidation of **9** by the Dess−Martin procedure[18,19] afforded 15-oxo-grandiflorenic acid (**1**). The ^1H and ^{13}C NMR spectra of this compound were also different from those given for licamichauxiioic acid A (Table 1), indicating again that its structure was erroneous.

The structure **3** assigned to licamichauxiioic acid B must also be incorrect, because the NMR data are not in accordance with this structure, which, in addition, violates Bredt's well-known rule, which basically postulates that in bridged bicyclic compounds the existence of double bonds at the bridgehead positions is impossible in small ring systems.[20] This does not apply when the rings are large enough, for example, in bicyclo[4,2,1]non-1(8)-ene. Other criteria about the stability of bridgehead double bonds are the Fawcett[21] and Wiseman[22,23] proposals. For reviews on this topic see ref 24.

The spectroscopic data of licamichauxiioic acid B (**3**), given by the authors, i.e., UV, IR, ^1H and ^{13}C NMR, and 2D NMR data (COSY, HMQC, and HMBC)[7] were not in concordance with the structure given. Thus, the chemical shifts assigned to the signals at δ_C 35.7 for C-9 and 33.9 for C-11 have values that are too low and too high, respectively, for a compound with this type of structure. For example, in a compound with structure **14** the corresponding resonances are at δ_C 52.4 (C-9) and 18.1 (C-11).[25] Moreover, in the ^1H NMR spectrum of licamichauxiioic acids A and B, **1** and **3**, the resonances of the proton of the endocyclic double bond, H-9 and H-14, respectively, have a similar value, δ_H 5.45 and 5.44, which does not explain the differences in structures assigned to these compounds. In addition, the signal at δ_H 2.80 assigned to H-9 in **3** is too deshielded for this type of hydrogen and is more characteristic of H-13 in *ent*-kaur-16-ene derivatives.[25] As in the case of licamichauxiioic acid A (**1**), the value assigned to the resonance of C-8 (δ 61.9), in the ^{13}C NMR spectrum of licamichauxiioic acid B (**2**), δ 61.7, is too high for this type of structure. Moreover, these similar resonance values of C-8 in both acids do not explain the structural differences proposed for them. Thus, we are of the opinion that the structure of licamichauxiioic acid B (**3**) is also incorrect.

In conclusion, in this work we reported that the structures assigned to licamichauxiioic acids A and B must be erroneous. In addition, we described a useful method for the separation of grandiflorenic and grandiflorolic acids, **7** and **13**, and showed that the Dess−Martin reaction is a good alternative to the Swern reaction in the oxidation of allylic alcohols.

Experimental Section

General Experimental Procedures. Column chromatography was performed on Si gel 0.063−0.2 mm. ^1H and ^{13}C NMR spectra were obtained on a Bruker AMX2-500 operating at 500 MHz for ^1H and 125 MHz for ^{13}C. Low- and high-resolution MS were taken at 70 eV (probe) in a Micromass Autospec spectrometer. Dry column chromatography was performed on Merck Si gel 0.02−0.063 mm.

Oxidation of 7 and 11 with Selenium Dioxide. The mixture of methyl esters **7** and **11** (120 mg), dissolved in 18 mL of dioxane−H_2O (3:1), was treated with SeO_2 (135 mg) and left to stir for 5 h at room temperature. A saturated solution of NaCl was added and the solution extracted with EtOAc. After drying and removal of the solvent, the residue was purified by chromatography on Si gel. Elution with petroleum ether−EtOAc (9:1) afforded 15α-hydroxy-*ent*-kaur-9(11),16-dien-19-oic acid methyl ester (**8**) (55 mg): ^1H NMR (500 MHz, $CDCl_3$) δ 0.96 (3H, s, H-20), 0.98 (1H, td, J = 13 and 4 Hz, H-3β), 1.19 (3H, s, H-18), 1.32 (1H, dd, J = 11 and 1.5 Hz, H-14), 1.47 (1H, m, H-6), 1.79 (1H, dd, J = 11 and 6 Hz, H-14), 2.14 (1H, ddd, J = 13, 2 and 1.6 Hz, H-3α), 2.40 (2H, m, H-2 and H-7), 2.75 (br s, H-13), 3.64 (3H, s, −OMe), 4.11 (1H, s, H-15), 5.19 and 5.20 (each 1H, s, H-17), 5.31 (1H, t, J = 4 Hz, H-11); EIMS m/z 330 $[M]^+$ (63), 315 (65), 312 (91), 297 (39), 271 (48), 255 (80), 253 (56), 237 (90), 212 (21), 197 (41), 183 (43), 172 (91), 159 (41), 157 (43), 145 (54), 143 (52), 105 (73), 91 (100); HREIMS m/z 330.2192 (calcd for $C_{21}H_{30}O_3$, 330.2194. Further elution gave 15α-hydroxy-*ent*-kaur-16-en-19-oic acid methyl ester (**12**) (42 mg).

Swern Oxidation of 8. Oxalyl chloride (90 μL) was added to dry DCM (7.2 mL) in a dry flask under N_2, and the solution was cooled to −72 °C. After 10 min DMSO (120 μL) was added and allowed to react for 10 min, before addition of the 15α-hydroxy derivative **8** (50 mg) in dry DCM (4 mL). The reaction mixture was stirred at −72 °C for a further 45 min. Di-isopropylethylamine (0.60 mL) was added dropwise, and the reaction mixture was then allowed to warm to room temperature. The solvent was removed under reduced pressure and the residue purified by chromatography. Elution with *n*-hexane−EtOAc (9:1) gave 15-oxo-*ent*-kaur-9(11),16-dien-19-oic acid methyl ester (**2**) (18 mg): ^1H NMR (500 MHz, $CDCl_3$) δ 0.96 (3H, s, H-20), 1.02 (1H, td, J = 13 and 5 Hz, H-3β), 1.17 (1H, td, J = 13 and 5 Hz, H-1β), 1.24 (3H, s, H-18), 1.42 (1H, m, H-2), 1.68 (2H, br s, H-14), 1.99 and 2.20 (each 1H, m, H-6), 2.09 (1H, m, H-1), 2.61 (1H, ddd, J = 13, 5 and 2.6 Hz, H-12), 2.94 (1H, br s, H-13), 3.64 (3H, s, -OMe), 5.43 and 5.90 (each 1H, s, H-17), 5.51 (1H, t, J = 3.5 Hz, H-11); ^1H NMR (500 MHz, C_6D_6) δ 0.93 (1H, td, J = 13 and 5 Hz, H-3β), 1.10 (3H, s, H-20), 1.21 (1H, td, J = 12 and 5 Hz, H-1β), 1.29 (3H, s, H-18), 1.39 and 1.40 (each 1H, s, H-14), 1.67 and 2.03 (each 1H, m, H-7), 1.73 (1H, dt, J = 13 and 4 Hz, H-1α), 1.91 (1H, ddd, J = 13, 5 and 2 Hz, H-12), 2.20 (1H, dt, J = 13 and 1.5 Hz, H-3), 2.50 (2H, m, H-6 and H-13), 3.32 (3H, s, −OMe), 5.05 and 6.01 (each 1H, s, H-17), 5.34 (1H, t, J = 3.5 Hz, H-11); EIMS m/z 328 $[M]^+$ (18), 313 (12),

296 (5), 169 (19), 268 (19), 253 (79), 173 (100), 233 (12); HREIMS *m/z* 328.2054 (calcd for $C_{21}H_{28}O_3$, 328.2038).

Oxidation of Grandiflorenic Acid (6) with Selenium Dioxide. Treatment of **6** (35 mg) with SeO_2 as described above for **7** afforded **9** (24 mg): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.98 (1H, td, J = 13.3 and 4.0, H-3β), 1.05 (3H, s, H-20), 1.17 (1H, td, J = 13.3 and 4.1 Hz, H-1β), 1.25 (3H, s, H-18), 1.32 (1H, d, J = 10.9 Hz, H-14), 1.48 (1H, m, H-2), 1.64 (1H, dd, J = 11.5 and 2.8 Hz, H-5), 1.68 (1H, m, H-7), 1.80 (1H, dd, J = 10.9 and 5.4 Hz, H-14), 2.15 (1H, dt, J = 13.3 and 3 Hz, H-3α), 2.39 (1H, ddd, J = 17.1, 4.3 and 2.7 Hz, H-12), 2.46 (1H, m, H-6), 2.75 (1H, br s, H-13), 4.11 (1H, s, H-15), 5.19 and 5.21 (each 1H, s, H-17), 5.32 (1H, t, J = 3.4 Hz, H-11); EIMS *m/z* 316 $[M]^+$ (45), 301 (34), 298 (10), 283 (25), 270 (11), 255 (40), 237 (29), 232 (10), 229 (6), 209 (12), 199 (16), 197 (18); HREIMS *m/z* 316.2033 (calcd for $C_{20}H_{28}O_3$, 316.2038).

Dess−Martin Oxidation of 9. A solution of the alcohol **9** (20 mg) in DCM (3 mL) was treated with the Dess−Martin reagent (30 mg) under N_2. The reaction was stirred for 3 h and then poured into an aqueous solution of sodium thiosulfate. Extraction with DCM in the usual way and chromatography of the extract, eluting with petroleum ether−EtOAc (7:3), afforded 15-oxo-*ent*-kaur-9(11),16-dien-19-oic acid (**1**) (14 mg): white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.06 (3H, s, H-20), 1.18 (1H, td, J = 13 and 4.5 Hz, H-3β), 1.28 (1H, m, H-1), 1.31 (3H, s, H-18), 1.45 (1H, m, H-2), 1.67 (2H, br s, H-14), 2.62 (1H, dt, J = 13 and 5 Hz, H-12), 2.94 (1H, br s, H-13), 5.43 and 5.91 (each 1H, s, H-17), 5.52 (1H, t, J = 3.5 Hz, H-11); ^{1}H NMR (500 MHz, C_6D_6) δ 0.87 (1H, td, J = 13 and 4 Hz, H-3β), 1.16 (3H, s, H-20), 1.17 (1H, td, J = 12 and 5 Hz, H-1β), 1.27 (3H, s, H-18), 1.36 (2H, br s, H-14), 1.62 and 2.31 (each 1H, m, H-7), 1.69 (1H, dt, J = 13 and 5 Hz, H-1α), 1.84 (1H, ddd, J = 13, 5 and 2 Hz, H-12), 2.15 (1H, dt, J = 13 and 1.5 Hz, H-3α), 2.25 (1H, m, H-12), 2.44 (1H, m, H-6) and 2.47 (1H, br s, H-13), 5.03 and 6.16 (each 1H, s, H-17), 5.30 (1H, t, J = 3.5 Hz, H-11); EIMS *m/z* 314 $[M]^+$ (24), 299 (16), 296 (5), 268 (21), 253 (78), 219 (21), 218 (11), 203 (14), 197 (24), 173 (100); HREIMS *m/z* 314.1869 (calcd for $C_{20}H_{26}O_3$, 314.1882).

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