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TWO NEW TRITERPENES AND OTHER CONSTITUENTS FROM *LUPINUS VARIUS* AND *LUPINUS HARTWEGII*

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تم فصل مركبين جديدين من نوع ثلاثى التربينات وهما ثنائى ميثيل سراتاجينات واستيل ثنائى ميثيل سراتاجينات بالإضافة إلى ستة مركبات معروفة ومشتقة من نواة الايزوفلافون وهى جينستين، ٢'-هيدروكسى جينستين ، لوبينالبين أ ، ج ، هـ ، ولوتيون بالإضافة إلى بيتاسيتوستيرول جلوكوزيد وسيرنجين وذلك من خلاصة الكلوروفورم للسيقان والأوراق الخاصة بالنباتين موضوع البحث.
هذا وقد تم التعرف على هذه المركبات بواسطة الطرق المختلفة للتحليل الكيماوى والطيفى.

Two new genuine triterpenes previously semisynthesized and given the names dimethyl serratagenate and acetyl dimethyl serratagenate, besides three known coumaronochromones named lupinalbins A, C and E, in addition to three common Lupin isoflavones known as genistein, luteone and 2´-hydroxy genistein as well as, the more frequent ß-sitosterol glucoside and the less frequent syringin were isolated from the chloroform fraction of the aerial parts of L. varius.

Moreover, the same compounds were almostly detected by co-chromatography with the corresponding fraction obtained from L. hartwegii. The identification of these compounds was based on different methods of chemical and spectral evidences.

INTRODUCTION

In a recent work[1] we have reported the alkaloidal constituents from both seeds of *Lupinus varius* L. ssp. orientalis Franco et Silva (= *L. digitatus Forssk, L. Pilosus* L., *L. hispanicus* and *L. microanthus*) known locally as Termis esh. shytann[2] and *Lupinus hartwegii* Lindl (= *L. maxicanus*) known popularly as Termis al-zuhoor. The genus *Lupinus* is also reputed of a number of constitutive 5-hydroxylated isoflavones which are considered as potent inhibitors for many potentially pathogenic fungi.[3-6] In *Lupinus* leaves, constitutive fungitoxic isoflavones appear to replace the induced isoflavonoids known as phytoalexins which occur as antimicrobial defence compounds in the tissues of a great many *Papilionate legumes*.[7]

In a further interest in the consituents of these two plants, this work describes the isolation of six known isoflavone derivatives, two new oleanene triterpenes besides the common ß-sitosterol glucoside and the less frequent syringin for the first time from the two plants under investigation.

EXPERIMENTAL

General

MPS: Uncorr., EI-MS on MAT 311A, 70ev. ^{1}H-NMR and ^{13}C-NMR in $CDCl_3$ (unless otherwise mentioned) at 400 and 100 MHz respectively with TMS as internal standard. UV with Perkin-Elmer model 550 spectrophotometer. TLC with precoated silica gel sheets (Aluminium foil, E. Merck). CC with SiO_2 (70-230 mesh E. Merck); Whatman No 3 filter sheets for PPC using 60% acetic acid in water as solvent system. The following solvent systems were used: 1) $CHCl_3$-MeOH (95:5), 2) $CHCl_3$-MeOH (85:15).

Plant material

Seeds of *L. varius* were collected from plants growing in Sinai near El-Arish, Egypt in April (1992). Seeds of *L. hartwegii* were kindly supplied by Prof. Dr. N. El-Keltawy (Dept. of

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Horticulture, Faculty of Agriculture, Assiut Univ., Assiut, Egypt). For enrichement, both sources were cultivated every year at the Experimental Station of Al-Azhar Univ., Assiut, Egypt from October (1992) till April (1996). Species were kindly identified by Prof. Dr. A. Fayed (Dept. of Botany, Faculty of Science, Assiut Univ., Assiut, Egypt).

Extraction and isolation

4 kg of dried aerial parts (stems and leaves) of *L. varius* were powdered and extracted with alcohol 70%. The conc. alcoholic extract (72 g) was diluted with distilled water and extracted successively and exhaustively with n-hexane, $CHCl_3$ and finally with n-BuOH. 2 kg of *L. hartwegii* were monitored similarly to afford a chloroform fraction of about 10 g. The TLC pattern of the chloroform fraction of the two plants was very similar in various solvent systems indicating presence of the same constituents. The $CHCl_3$ fraction of *L. varius* (25 g) was fractionated on SiO_2 CC using $CHCl_3$-MeOH gradient. Elution with $CHCl_3$-MeOH (98:2) afforded compound **1** and further fractions afforded **2**. Elution with $CHCl_3$-MeOH (97:3) afforded a mixture of **3,4** from which **4** (major) was obtained by repeated crystallization (MeOH) and the mother liquor was subjected to PPC using 60% HOAc to afford both **3** and **4**. Elution with $CHCl_3$-MeOH (96:4) afforded a mixture of **5** and **6** which were isolated by extracting their dry residue (about 300 mg) with ether where **6** was insoluble. While further column fractions gave pure **6**. Further elution with $CHCl_3$-MeOH (96:4) gave a mixture of flavonoids which are still under investigation. Elution with $CHCl_3$-MeOH (94:6) afforded **7** and **8** as a mixture with **8** as a major compound and was obtained by crystallization from MeOH. The remaining mother liquor was used for final separation of **7** with PTLC using $CHCl_3$-MeOH (93:7) as solvent system. Elution with $CHCl_3$-MeOH (90:10) afforded **9** and further fractions gave **10**.

Partial saponification of 8: 10 mg of **8** were refluxed with alcoholic KOH (7%, 20 ml) for 8 hours. The solution was then neutralized; 15 ml of distilled water were added and alcohol was removed. The monomethyl ester of **8** was extracted with $CHCl_3$ to give a compound identical with authentic sample of 28 methyl serratagenate (mmp and co-chromatography).

Compound 1: "Lupinalbin E". Colourless needles (10 mg), mp 256-258°C (MeOH) having yellowish flourescence in UV. R_F 0.55 (system 1). UV λ_{max} nm: MeOH: 216, 260, 284, 304 (sh), 337; NaOAc: 255 (sh), 267, 295, 303 (sh), 347; NaOAc/H_3BO_3: 216, 260, 284, 304 (sh), 336; $AlCl_3$ 223, 235 (sh), 270, 290, 325 (sh), 383; NaOMe: 253 (sh), 267, 295, 347. EI-MS m/z (rel. int%) 368 (M^+, 100), 339 (3), 335 (20), 311 (5), 310 (50), 309 (95; M-59), 298 (16), 297 (16), 296 (19), 281 (4), 59 (40). ^{1}H-NMR (δ ppm): 6.38 (1H, d, J= 2.1 Hz, H_6); 6.61 (1H, d, J= 2.1 Hz, H_8); 6.90 (1H, d, J= 8.3 Hz, $H_{5'}$); 7.75 (1H, d, J= 8.3 Hz, $H_{6'}$); 3.4 (2H, m, $H_{1''a}$ and $H_{1''b}$); 4.84 (1H, dd, J= 9.4, 8.3 Hz, $H_{2''}$); 1.28, 1.32 (each 3H, s, $Me_{4''}$ and $Me_{5''}$); 10.8 (1H, brs, C_7-OH); 12.98 (C_5-OH).

Compound 2: "Lupinalbin C". Colourless prisms (7 mg), mp 260-262°C (MeOH). R_F 0.48 (system 1). UV λ_{max} nm: MeOH: 243 (sh), 260, 287, 333 (sh). The MeOH spectrum is not affected by NaOAc; $AlCl_3$: 215, 243, 270, 289, 332, 376; NaOMe: 271, 315 (sh). EI-MS m/z (rel. int%) 368 (M^+, 61), 335 (30), 310 (60), 309 (100; M-59), 282 (6), 281 (5), 59 (65). ^{1}H-NMR (δ ppm): 6.59 (1H, s, H_8); 7.15 (1H, d, J= 2.1 Hz, $H_{3'}$); 7.04 (1H, dd, J= 8.3, 2.1 Hz, $H_{5'}$); 7.84 (1H, d, J= 8.3 Hz, $H_{6'}$); 3.2 (2H, m, $H_{1''a}$ and $H_{1''b}$); 4.9 (1H, dd, J= 9.0, 8.4 Hz, $H_{2''}$); 1.27, 1.33 (each 3H, s, $Me_{4''}$ and $Me_{5''}$); 9.7 (1H, brs, $C_{4'}$-OH); 13.0 (C_5-OH).

Compound 3: "Genistein". Pale yellow needles (5 mg), mp 302-304°C (MeOH). R_F 0.39 (system 1). UV λ_{max} nm: MeOH: 262, 297 (sh), 327 (sh); NaOAc: 271, 328; NaOAc/H_3BO_3: 262, 335 (sh); $AlCl_3$: 273, 312 (sh), 366; $AlCl_3$/HCl: 273, 312 (sh), 366; NaOMe: 276, 337 (sh). ^{1}H-NMR (CD_3OD, δ ppm): 6.1 (1H, d, J= 2 Hz, H_6); 6.23 (1H, d, J= 2 Hz, H_8);

6.7 (2H, dd, J= 8.5, 2.1 Hz, $H_{3',5'}$); 7.2 (2H, dd, J= 8.5, 2.1 Hz, $H_{2',6'}$).

Compound 4: "Luteone". Pale yellow powder (60 mg). Redish purple in UV light. mp 225-227°C (MeOH). R_F 0.39 (system 1). UV λ_{max} nm: MeOH: 266, 290 (sh); NaOMe: 224, 279, 337; $AlCl_3$: 276, 316 (sh); $AlCl_3$/HCl: 277, 313 (sh), NaOAc: 271, 340 (sh); NaOAc/H_3BO_3: same as MeOH spectrum. ^{1}H-NMR (δ ppm): 8.14 (1H, s, H_2); 6.53 (1H, s, H_8); 6.48 (1H, d, J= 2.3 Hz, $H_{3'}$); 6.44 (1H, dd, J= 8.8, 2.3 Hz, $H_{5'}$); 7.12 (1H, d, J= 8.8 Hz, $H_{6'}$); 3.37 (2H, m, $H_{1''a}$ and $H_{1''b}$); 5.28 (1H, brt, J= 7 Hz, $H_{2''}$); 1.65, 1.79 (each 3H, s, $Me_{4''}$ and $Me_{5''}$); 13.02 (C_5-OH).

Compound 5: "Lupinalbin A". Colourless needles (45 mg). Yellowish flourescent in UV light, mp 310-312°C (MeOH). R_F 0.3 (system 1). UV λ_{max} nm: MeOH: 257, 283 (sh), 301 (sh), 333 (sh); NaOAc: 260, 291, 346; NaOAc/H_3BO_3: same as MeOH spectrum; $AlCl_3$: 268, 285 (sh), 302 (sh) 322 (sh); NaOMe: 272, 305, 360. ^{1}H-NMR (DMSO-d_6, δ ppm): 6.28 (1H, d, J= 2.1 Hz, H_6); 6.53 (1H, d, J= 2.1 Hz, H_8); 6.85 (1H, dd, J= 8.38, 2.1 Hz, $H_{5'}$); 7.08 (1H, d, J= 2.1 Hz, $H_{3'}$); 7.7 (1H, d, J= 8.38; $H_{6'}$); 9.9, 10.9 (each 1H, s, two phenolic OH), 12.9 ($C_{5'}$-OH; chelated). ^{13}C-NMR C_2-C_{10}: 154.9, 121.3, 178.3, 162.3, 99.9, 163.7, 95.2, 156.4, 103; $C_{1'}$-$C_{6'}$: 113.5, 150.2, 99.0, 156.4, 114, 121.2.

Compound 6: "2-hydroxygenistein, 5,7,2´,4´-tetrahydroxyisoflavone". Pale yellow needles (250 mg), mp 270-272 (MeOH). R_F 0.26 (system 1). UV λ_{max} nm: MeOH: 261, 288 (sh), 339; NaOMe 274, 322; NaOAc: 270, 331; NaOAc/H_3BO_3: as MeOH spectrum; $AlCl_3$: 267, 310 (sh), 361; $AlCl_3$/HCl: 267, 310 (sh), 361. EI-MS m/z (rel. int%) 286 (100), 269 (22), 258 (5), 229 (6), 216 (6), 160 (4.8), 152 (73), 134 (45), 124 (13), 69 (35), 51 (21.3). ^{1}H-NMR (CD_3OD, δ ppm): 6.22 (1H, d, J= 2 Hz, H_6); 6.4 (1H, d, J= 2 Hz, H_8); 6.36 (1H, d, J= 2.05 Hz, $H_{3'}$); 6.39 (1H, dd, J= 8, 2.05 Hz, $H_{5'}$); 7.2 (1H, d, J= 8.0 Hz, $H_{6'}$); 8.1 (1H, s, H_2). ^{13}C-NMR C_2-C_{10}: 155.6, 121.5, 181.5, 162.6, 99.2, 165.2, 93.8, 158.7, 105; $C_{1'}$-$C_{6'}$: 109.7, 156.7, 103.2, 159.1, 107.1, 132.1.

Compound 7: "Acetyl dimethyl serralagenate" (1.5 mg), mp 272-274°C (MeOH). EI-MS m/z (rel. int%) 556 (3), 496 (30), 481 (5), 306 (80), 249 (5), 247 (35), 246 (70), 233 (22), 187 (100).

Compound 8: "Dimethyl serratagenate" (20 mg), mp 203-205°C (MeOH). EI-MS m/z (rel. int%) 514 (22.7), 496 (12), 483 (13.6), 455 (22.7), 454 (22), 438 (22.6), 395 (11), 377 (4), 316 (4), 306 (50), 247 (60), 207 (31.5), 187 (100), 133 (11.1), 75 (31.2). ^{1}H-NMR (CD_3OD-d_6, δ ppm) 0.78, 0.88, 0.94, 0.97, 1.14, 1.26 (each 3H, s, 6Me groups); 2.96 (1H, dd, J= 14, 6.3 Hz, H_{18}), 3.48 (1H, m, $H_{3\alpha}$); 3.66 (6H, 2COOMe), 5.22 (1H, t, J= 2.5 Hz, H_{12}). ^{13}C-NMR C_1-C_{30}: 38.2, 27.9, 79.8, 39.8, 56.8, 19.9, 33.2, 39.9, (C_9 obscured by CD_3OD signals), 37.9, 24.5, 123.3, 145.9, 42.3, 30.0, 24.6, (C_{17} obscured by CD_3OD signals), 42.9, 40.6, 42.3, 29.2, 34.2, 28.8, 16.3, 15.9, 18.1, 26.4, 181.2, 185.4, 19.6, and 2COO<u>Me</u> at 52.3.

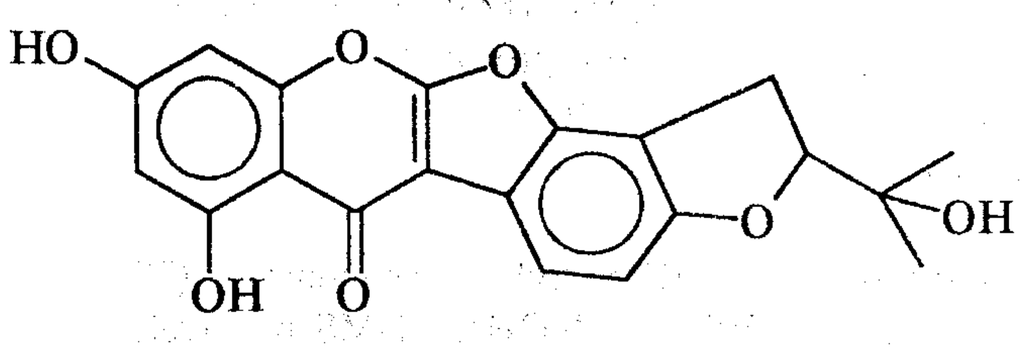
Compound 9: Spherical crystals of mp 275-277°C not depressed by authentic sample of ß-sitosterol glycoside. Co-chromatography with authentic sample of ß-sitosterol glucoside (system 2) showed also their identity.

Compound 10: mp 191-193. The compound was identified as syringin by direct authentication (mmp and co-chromatography on system 2).

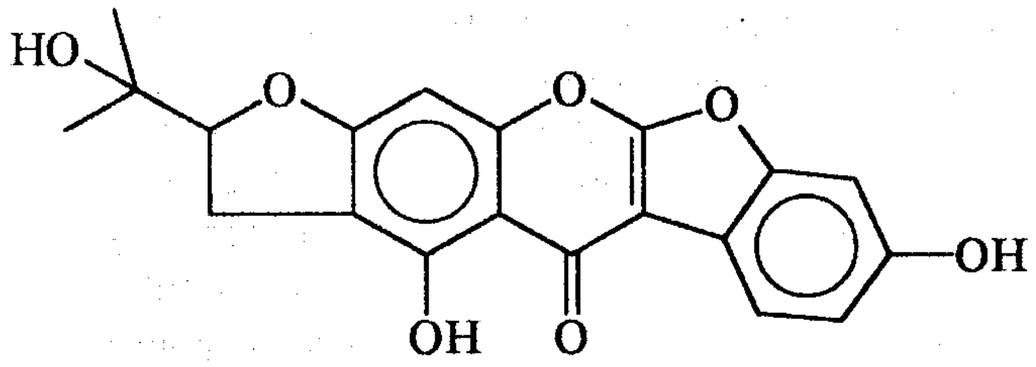
RESULTS AND DISCUSSION

The isoflavone character of **3** was evident from its UV data (λ_{max} nm: MeOH 262, 297 (sh)) which was virtually superimposible on those reported for genistein [5,8] showing an evident band π bathochromic shifts with NaOAc and $AlCl_3$ indicating free phenolic hydroxyl groups in positions 7 and 5 respectively.[5] More confirmation was achieved through its ^{1}H-NMR which clearly indicates its identity to genistein structure (see exp.).

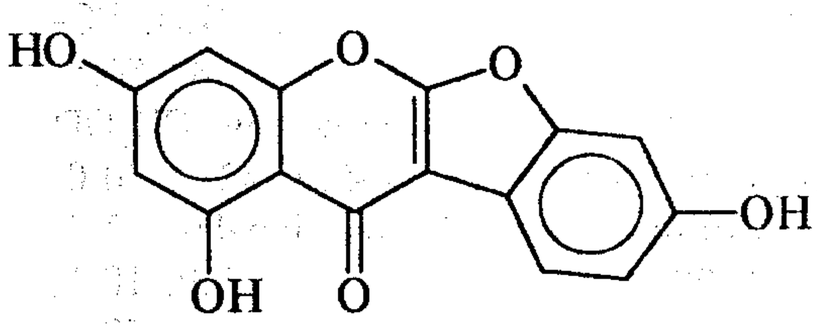
Similarly the isoflavone nature of **6** was clear from its spectral data (UV, ^{1}H-NMR, MS



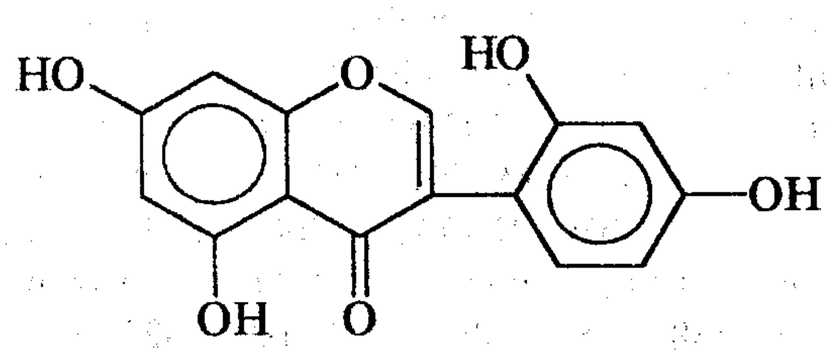
Compound **1**: Lupinalbin E



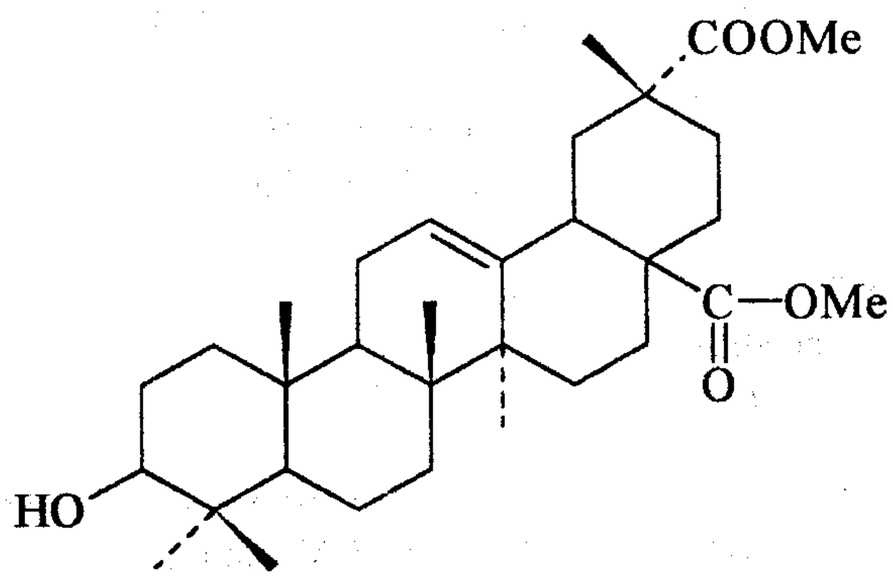
Compound **2**: Lupinalbin C



Compound **5**: Lupinalbin A



Compound **6**: 2'-Hydroxygenistein



Compound **8**: Dimethyl serratagenate

Structures of some of the isolated compounds

and ^{13}C-NMR, see exp.). Bathochromic shifts with NaOAc and $AlCl_3$ were similar to those of **3** indicating identical oxygenation pattern in ring A. The MS spectrum of **6** exhibited [M^+] at m/z 286 and further RDA fragments of an isoflavone having two hydroxyl groups in both benzoyl and cinnamoyl moieties at m/z 152 and 134 respectively.[8] Moreover the MS spectrum showed a peak at m/z 269 [M-OH]$^+$ indicating that **6** is an isoflavone having 2´-hydroxyl group.[8]

Further inspection of the ^{1}H and ^{13}C-NMR of **6** confirmed a 2´,4´-hydroxylation in ring B and 5,7-hydroxylation in ring A. As such **6** was unambigiously identified as 2´-hydroxygenistein. Both genistein and its 2´-hydroxy derivative were reported repeatedly from all studied *Lupinus* species and seems to be common markers in genus *Lupinus*.[9] In addition, the two aforementioned isoflavones were isolated from many *Leguminoseae* plants.[9]

It is noteworthy to mention that 2´-hydroxygenistein is the major isoflavone in both *L. varius* and *L. hartwegii* and it was simply isolated from other admixed isoflavones of the two plants in concern by taking the advantage of its insolubility in ether.

The identification of **5** as an isoflavone like compound was initially based on its UV (MeOH) spectrum exhibiting a prominent maximum at λ_{max} 257 nm and other associated shoulder at λ_{max} 301 nm. Bathochromic shifts were also observed with NaOAc and $AlCl_3$.

The aromatic hydroxy signals in the ^{1}H-NMR spectrum of **5** were evident as two broad signals at δ 9.90 and 10.9 (each 1H, brs) and a sharp chelated one[10,11] at δ 12.9 (1H, sharp signlet, OH-5). The aromatic protons of **5** were also evident as two meta-coupled doublets at δ 6.28, 6.53 (each 1H, d, J= 2.1 Hz, H_6 and H_8 respectively) of 5,7 dioxygenated isoflavone and an ABX system at δ 6.85, 7.08, 7.7 for a 2´, 4´-dioxygenation.[12] However in comparison with the ^{1}H-NMR of **3** and **6**, the ^{1}H-NMR of **5** lacks the characteristic H-2 singlet of isoflavones. When the UV, MS and ^{1}H-NMR data are taken into account; compound **5** could be formulated as lupinalbin A or its isomeric trihydroxycoumestan (aureol).[13] The latter structure was ruled out since lupinalbin A has a chelated hydroxyl group (^{1}H-NMR and UV data). Further comfirmation was achieved from ^{13}C-NMR which is reported here for the first time (see exp).

Identification of compounds **1** and **2** as lupinalbin E and C respectively were easily deduced by comparison of their ^{1}H-NMR, MS and UV data with lupinalbin A, where both of them afforded [M^+] at m/z 368 and two major fragments at m/z 309 [M-59]$^+$ and m/z 59 which are indicative of a hydroxy isopropyl dihydrofurano attachment.[3] In compound **2** "lupinalbin C", the attachment was assigned to ring A due to lack of any bathochromic shift with NaOAc in comparison with its MeOH spectrum and from comparison of its ^{1}H-NMR with **5** where both of them showed identical oxygenation pattern in ring B. However **2** showed absence of the most upfield aromatic signals characteristic for H_6; hence; establishing the dihydrofurano attachment to be cyclized from C_6 to oxygen atom of C_7.

Conversely in **1** "lupinalbin E" which showed normal ring A substitution as in **5** (UV, ^{1}H-NMR) and only a pair of ortho coupled protons in the B-ring "each 1H, d, J= 8.3 Hz, $H_{5',6'}$); hence; establishing the dihydrofurano attachment to be cyclized from $C_{3'}$ to oxygen atom of $C_{4'}$. The last three coumaronochromones (2,2´-epoxyisoflavones) have been isolated once previously from roots of *L. albus*.[14]

Compound **4** was identified as 5,7,2´,4´-tetrahydroxy-6-(3,3-dimethyl allyl)-isoflavone "luteone" from its UV spectrum which showed absorption maxima at 266 and 290 (sh) and the ^{1}H-NMR singlet at δ 8.14 characteristic for H_2 of isoflavones. The upfield region of the ^{1}H-NMR of **4** showed signals at δ 1.65 and 1.79 (3H each, brs), δ 3.37 (2H, m, J= 8 Hz) and 5.28 (1H, brt, J= 7.0 Hz) indicating presence of 3,3-dimethyl allyl group. This was supported by MS spectrum of **4** which exhibited [M^+] and m/z 354 in addition to a characteristic fragment at m/z 299 (M-55) providing further confirmation for 3,3-dimethyl allyl group. Comparison of **4** with **6** showed absence of H_6, and similar oxygenation pattern in B ring indicating the presence of the isoprenyl unit at C_6 of **4**. This compound (major in *L. hartwegii*)

has been previously reported to be a common component in all investigated lupin leaves including *L. hartwegii*[15] and this represents its first report in *L. varius*.

The ^{1}H-NMR of **8** showed six sharp three proton singlets at δ 0.78-1.26 (each 3H, s, Mex6), two COOMe at δ 3.66 (6H, s), an α axial methine carbinolic proton geminal to ß-equatorial hydroxy function at δ 3.48 (1H, dd, J= 10.6, 4.5 Hz, $H_{3\alpha}$) and an olefinic proton at δ 5.22 (1H, triplet like, J= 2.5 Hz, H_{12}). These data together with the ^{13}C-NMR signals at δ 52.3, 79.8, 123.3, 145.9, 181.2 and 185.4 are indicative of an olean-12-ene skeletone having 3ß-hydroxy function and two methoxy carbonyl groups.[16-19]

The EI-MS of **8** further comfirmed this conclusion by showing [M^+] at m/z 514 and typical RDA fragments[20] at m/z 306 and 207 pertaining to upper part of the triterpene skeleton having two COOMe and its lower part having the 3-ß-hydroxyl group respectively.

The ^{1}H-NMR downfield shifted methyl signal at δ 1.26 indicated that its geminal methyl group is oxidized[21,22] and as such locating one COOMe on C_{20}. Its chemical shift is in agreement with C_{29} carboxy triterpenes.[23] This was confirmed by ^{13}C-NMR upfield signal at δ 19.6 assigned for C_{30} methyl group geminal to an α-equatorial carbomethoxyl function at C_{29}.[19,24,25] The second carbomethoxy function was located at C_{28} since C_{27} position was excluded due to absence of strong M-59 and or M-60 as occurs in C_{27} carboxy compound due to allylic activation[20,26,27] and due to normal C_{14} resonance in comparison with related compunds.[19] From these data compound **8** was identified as dimethyl serratagenate[28], rather than its isomeric dimethyl spergulagenate.[29] Final confirmation was achieved by saponification of **8** where a compound identical to authentic 28-methyl serratagenate[30] (mmp and co-chromatography was obtained).

Compound **7** was isolated in trace amounts. Its EI-MS is similar to **8** except for [M^+] which was shifted to m/z 556 and the peak at m/z 207 in **8** was shifted to m/z 249 indicating an acetate function at C_3. Its mp is also matched with acetyl dimethyl serratagenate.[28] Its small amount percluded further analysis. Both **7** and **8** (detected in high concentration in hexane fraction) were previously semisynthesized from serratagenic acid isolated from *clerodendron serratum* (*verbenacea*)[28] and this is their first report as new genuine natural products.

Compound **9** was identified as the common ß-sitosterol glucoside (mmp and co-chromatography) while **10** as syringin by direct authentication (mmp. and co-chromatography). The last two compounds are reported for the first time in genus *Lupinus*.

It is worthy to mention that these compounds were isolated from L. varius and except for Lupinalbin E (compound **1**), other spots were detected after a rapid fractionation of the chloroform fraction of *L. hartwegii* followed by TLC comparison with samples isolated from *L. varius*. However, the hexane fraction of *L. varius*, and *L. hartwegii* were identical in their triterpenes content. Also the n-BuOH fraction of *L. varius* showed four flavonoidal glycosides while that of *L. hartwegii* showed five; four of them are chromatographically identical to those of *L. varius*. Detailed work on these constituents is in progress.

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