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VELUTINIC ACID, A NEW FRIEDELANE DERIVATIVE FROM *XYLOSMA VELUTINA* (FLACOURTIACEAE)

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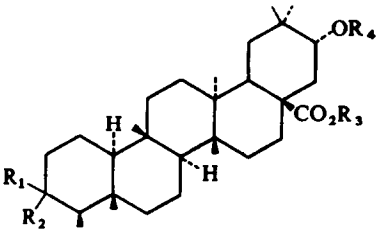
Key Word Index—*Xylosma velutina*; Flacourtiaceae; velutinic acid; new friedelane derivative; flavonoids; genkwanin; velutin.

The pantropical genus *Xylosma* numbers in excess of one hundred species [1], but previous phytochemical [2] and pharmacological [3] work has been quite limited.

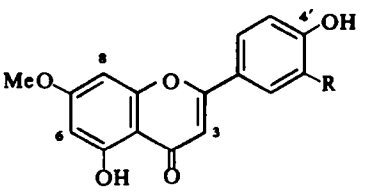
Chromatography of the chloroform soluble fraction from the twigs, leaves and inflorescence of *Xylosma velutina* afforded three pure components; two known flavonoids and a new triterpene acid.

Velutinic acid (1) crystallized from $CHCl_3$, mp 288–290°, and the physical and chemical data (see Experimental) indicated the presence of carboxyl, hydroxyl and ketone functions with a molecular weight of 472.

The general appearance of the MS fragmentation patterns of the substance and its derivatives, particularly the strong peak at *m/e* 273 in velutinic acid shifting to *m/e* 275 in the dihydro derivative, were consistent with the presence of a friedelane nucleus [5, 6]. From the fragmentation patterns of velutinic acid (1) and dihydrovelutinic acid (2), the ketone group could be located in ring A or ring B. There was no M^+-15 peak in the MS of velutinic acid; however significant peaks were observed at *m/e* 409 (M^+-H_2O − CO_2H) and 408 (M^+-H_2O − HCO_2H), and related fragments were also found in the MS of methyl velutinate (3) and acetyl methyl velutinate (4), indicating that there was a carboxyl group at position 17 [5]. The remaining functional group (hydroxyl) is situated in ring E of velutinic acid, as indicated by the mass shifts in the corresponding fragments of 1, 3 and 4.



1 R_1, R_2 = =O; R_3 = H; R_4 = H
2 R_1 = α-H; R_2 = β-OH; R_3 = H; R_4 = H
3 R_1, R_2 = =O; R_3 = Me; R_4 = H
4 R_1, R_2 = =O; R_3 = Me; R_4 = COMe
5 R_1 = α-H; R_2 = β-OH; R_3 = H; R_4 = COMe
6 R_1 = α-H; R_2 = β-OH; R_3 = Me; R_4 = COMe



7 R = OMe
8 R = H

The PMR spectrum of **4** showed a *dd* at δ 4.72 (J = 12 and 3 Hz) integrating for one proton. This observation ruled out the possibility of a hydroxyl group at C-19, and from the coupling constants the methine proton should be axial. Comparison of PMR data of **3** and **4** permitted a determination of the position of the hydroxyl group. Acetylation deshielded two methyl singlets (at C-20) from δ 0.97 to 1.04 and from δ 1.10 to 1.16. Since each methyl group was affected almost equally the hydroxyl group is located at C-21 in an α-configuration.

Reduction of velutinic acid with $NaBH_4$ afforded an axial alcohol [7], and confined the ketone group to ring A [8].

The chemical shift of the C-4 methyl group in friedelane derivatives having a ketone at C-3 is δ 0.88. In the absence of a C-3 ketone group, the C-4 methyl group is shifted upfield to δ 0.75 [8]. According to the average chemical shift, δ 0.85, of the C-4 methyl group in **3** and **4**, the ketone group can be placed at C-3.

Independent evidence for the placement of the ketone at C-3 was derived from an examination of the PMR spectrum of the acetylated dihydro derivative **5**. Introduction of a β-acetoxy group in the friedelane skeleton causes a downfield shift of the C-5 methyl group of approximately 11.0 Hz, and an upfield shift of the C-4 methyl of approximately 3.6 Hz in comparison with a ketone group at C-3. An α-acetoxy group at C-3 causes a downfield shift of the C-5 methyl of *ca* 4.0 Hz and an upfield shift of the C-5 methyl of approximately 8.4 Hz [7, 8].

The PMR spectral data of methyl velutinate (**3**), acetyl methyl velutinate (**4**), acetyldihydrovelutinic acid (**5**), and acetyl methyl dihydrovelutinate (**6**) indicate that replacement of the group at C-3 by an acetoxy group causes an average downfield shift of 12.9 Hz on the C-5 methyl group, and an average upfield shift on the C-4 methyl of 1.8 Hz. These data clearly establish the acetoxy group to be at C-3 and in a β-configuration. The original keto group is therefore confirmed to be at C-3 and velutinic acid has the structure **1**.

The two flavonoid derivatives obtained were identified as velutin (3',7-dimethoxy-4',5-dihydroxyflavone) (**7**) and genkwanin (4',5-dihydroxy-7-methoxyflavone) (**8**) by direct comparison with authentic samples.

EXPERIMENTAL

Mp's were determined by means of a Kofler hot plate and are uncorr. PMR spectra were recorded in $CDCl_3$ or DMSO-d_6 solns at 60 MHz. Tetramethylsilane was used as an internal standard and chemical shifts are reported in δ units. Column chromatography was carried out using Florisil, or Si gel PF-254.

Plant material. The leaves, twigs, inflorescences (fruiting) and stem bark used in this study were collected in Columbia during March 1974 and identified by Dr. R. E. Perdue, Jr. of the Medical Plant Resources Laboratory, Beltsville, Maryland. A voucher specimen is deposited in the Herbarium of the National Arboretum, U.S. Department of Agriculture, Washington, DC.

Extraction and fractionation. The dried and milled leaves, twigs and inflorescence (fruiting) of *Xylosma velutina* (Tul.) Tr. and Pl. (16.8 kg) were extracted with MeOH, and the combined extracts filtered and dried *in vacuo* to afford 2.22 kg of fraction A. A major portion (2.13 kg) of fraction A was dissolved in a mixture of MeOH–H_2O (9:1) and partitioned against petrol to give 155 g of petrol solubles (fraction B). The MeOH–H_2O extract was concnd to a syrup *in vacuo* and partitioned between $CHCl_3$ and H_2O to give 36 g of $CHCl_3$-soluble material (fraction C).

Chromatographic separation of fraction C. A major portion of fraction C (34.6 g) was chromatographed on Florisil (320 g) in a glass column (5.6 × 58 cm) eluting with $CHCl_3$. A total of 227 fractions (100 ml each) were collected as the solvent was progressively changed to increasingly polar mixtures of $CHCl_3$–MeOH, and finally to MeOH. Fractions 22–72 (13.7 g), eluted with $CHCl_3$–MeOH (19:1), were rechromatographed on a column (6 × 105 cm) of Si gel PF-254 (750 g), beginning with 1% MeOH in $CHCl_3$, and progressively changing the developing solvent to 20% MeOH in $CHCl_3$.

Isolation and identification of velutin (**7**). Pooled fractions 47–59 from the second column afforded a yellow crystalline substance from MeOH (43.3 mg), mp 225–227°; λ_{max}^{MeOH} nm(log ε): 241 (4.15), 226 (4.13), and 345 (4.26); λ_{max} (0.1N NaOH) nm(log ε): 232 (4.18), 260 (4.21), 298 (3.75) and 400 (4.45); λ_{max} ($AlCl_3$/HCl) nm: 262, 277, 295, 360, 387.5; λ_{max} (NaOAc) nm: 242.5, 252.5, 267.5, 350, 410 (*sh*); ν_{max}^{KBr} cm^{-1}: 3430 (*br*), 3080 (*w*), 3000 (*w*), 2920 (*w*), 1650 (*s*), 1595 (*s*), and 1500 (*s*); PMR: δ 3.83 (3H, *s*, —OMe), 3.91 (3H, *s*, —OMe), 6.57 (1H, *d*, J = 2.2 Hz, H-6), 6.78 (1H, *d*, J = 2.2 Hz, H-8), 6.93 (1H, *s*, H-3), 6.95 (1H, *d*, J = 8.9 Hz, H-5'), 7.57 (2H, *m*, H-2' and H-6'); MS, *m/e* (rel. int.): 314 (M^+, 100), 286 (12.1), 271 (9.5), 167 (13.8), 166 (2), 148 (6), 143 (11.2), 138 (3), 133 (6) and 95 (6). The spectral data indicated the compound to be 4',5-dihydroxy-3',7-dimethoxyflavone (velutin) (**7**). Direct comparison of the IR spectra of the isolate with reference velutin and mmp determination confirmed the identity.

Isolation and identification of genkwanin (**8**). Pooled fractions 128–240 from the second column afforded a yellow crystalline substance from $CHCl_3$ (63.3 mg), mp 282–284°; λ_{max}^{MeOH} nm(log ε): 267 (4.57) and 332 (4.64); λ_{max} (0.1N NaOH) nm(log ε): 230 (4.53), 252 (4.49), 265 (4.50), 298 (4.14), and 389 (4.89); λ_{max} ($AlCl_3$/1HCl) nm: 266.5, 300, 345, and 385; λ_{max} (NaOAc) nm 267, and 387.5; ν_{max}^{KBr} cm^{-1}: 3400–3000 (*br*), 1670 (*s*), 1610 (*s*), 1590 (*s*), 1500 (*s*), 830 (*s*); PMR: δ 3.87 (3H, *s*, —OMe), 6.36 (1H, *d*, J = 2.2 Hz, H-6), 6.74 (1H, *d*, J = 2.2 Hz, H-8), 6.82 (1H, *s*, H-3), 6.93 (2H, *d*, J = 8.9 Hz, H-3',5') and 7.95 (2H, *d*, J = 8.9 Hz, H-2',6'); MS, *m/e* (rel. int.): 284 (M^+, 100), 256 (24), 241 (10), 167 (9), 166 (9), 138 (12), 121 (5), 118 (7), and 95 (10). The spectral data indicated the compound to be 4',5-dihydroxy-7-methoxy-flavone (genkwanin) (**8**). Direct comparison of the IR spectra of the isolate with reference genkwanin and mmp determination confirmed the identity.

Isolation and characterization of velutinic acid (**1**). Pooled fractions 341–463 from the second column afforded a colorless crystalline substance from $CHCl_3$ (42 mg), mp 288–290°; $[\alpha]_D^{26}$ = −76° (conc = 0.5 in MeOH); ν_{max}^{KBr} cm^{-1}: 3470 (*br*), 3100 (*m*), 2920 (*s*), 1705 (*s*), 1685 (*s*), 1450 (*m*), 1380 (*m*), 1250 (*s*), 1175 (*m*), 1080 (*w*), 1035 (*w*), 1000 (*w*), 970 (*w*), 850, 780 (*w*), 740 (*w*) and 675 (*w*); PMR (DMSO-d_6): δ 0.62 (3H, *s*), 0.73 (3H, *d*, J = 6 Hz), 0.84 (3H, *s*), 0.93 (3H, *s*), 0.96 (3H, *s*), 1.05 (3H, *s*), 1.06 (3H, *s*) 3.40 (1H, *dd*, J = 12 and 3 Hz, H-16); MS, *m/e* (rel. int.): 472 (M^+, 100), 454 (52.8), 436 (64), 426 (16.8), 409 (30.3), 408 (60.8), 393 (31.2), 383 (16.8), 332 (41.6), 303 (18.4), 285 (23.2), 283 (20.2), 273 (17.6), 257 (25.6), 237 (17.6), 219 (33.6), 205 (24.8), 193 (32.0), 191 (41.6), 123 (68), and 121 (49.6).

Methylation of velutinic acid (**1**). Velutinic acid (**1**) was treated with CH_2N_2 (prepared from diazald) in Et_2O at room temp. overnight to yield methyl velutinate (**3**); MS, *m/e* (rel. int.): 486 (M^+, 100), 303 (28.2), 273 (47.4) and 205 (32.1), PMR ($CDCl_3$): δ 0.76 (3H, *s*, C-5 Me), 0.83 (3H, *d*, J = 6 Hz, C-4 Me), 0.92 (3H, *s*), 0.93 (3H, *s*), 0.97 (3H, *s*), 1.10 (3H, *s*), 1.20 (3H, *s*), and 3.7 (3H, *s*, —OMe).

Acetylation of methyl velutinate (**3**). Methyl velutinate (**3**) was treated with Ac_2O–Py (1:1) at room temp. overnight to afford acetyl methyl velutinate (**4**): MS, *m/e* (rel. int.): 528 (M^+, 0.8), 303 (2.4), 273 (5.7), and 205 (6.5), PMR ($CDCl_3$): δ 0.71 (3H, *s*, C-5 Me), 0.86 (3H, *d*, J = 6 Hz, C-4 Me), 0.91 (3H, *s*), 0.96 (3H, *s*), 1.04 (3H, *s*), 1.16 (3H, *s*), 1.21 (3H, *s*), 2.0 (3H, *s*, —OAc), 3.8 (3H, *s*,—OMe) and 4.72 ppm (1H, *dd*, J = 12 and 3 Hz, —CH—).

Borohydride reduction of velutinic acid (**1**). Velutinic acid (**1**) in MeOH was treated with $NaBH_4$ at room temp. for 30 min. The soln was evaporated and the residue worked-up in the usual way for organic material. The product was homogeneous by TLC

and was identified as dihydrovelutinic acid (2); MS, *m/e* (rel. int.): 474 (M^+, 16.7), 305 (21.7), 275 (17.2) and 207 (34).

Acetylation of dihydrovelutinic acid (2). Dihydrovelutinic acid (2) was treated with Ac_2O–Py (1:1) at room temp. for 12 hr to afford acetyl dihydrovelutinic acid (5); PMR ($CDCl_3$): δ 0.83 (3H, *d*, J = 6 Hz), 0.90 (3H, *s*), 0.95 (6H, *s*), 1.08 (3H, *s*), 1.16 (3H, *s*), 1.21 (3H, *s*), 2.00 (3H, *s*, —OAc) 2.02 (3H, *s*, —OAc), and 4.86 (2H, *m*).

Methylation of acetyldihydrovelutinic acid (5). Acetyl dihydrovelutinic acid (5) was treated with CH_2N_2 in Et_2O at room temp. overnight to afford acetyl methyl dihydrovelutinate (6); PMR ($CDCl_3$): δ 0.80 (3H, *d*), 0.92 (3H, *s*), 0.95 (6H, *s*), 1.07 (3H, *s*), 1.15 (3H, *s*), 1.20 (3H, *s*), 1.99 (3H, *s*, —OAc), 2.02 (3H, *s*, —OAc), 3.77 (3H, *s*, —OMe), 4.36 (1H, *s*, J = 12 and 3 Hz, H-16) and 4.76 (1H, *m*, H-3).

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MISE EN ÉVIDENCE DE DEUX TYPES CHIMIQUES CHEZ LE *CANNABIS SATIVA* ORIGINAIRE D'AFRIQUE DU SUD

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Key Word Index—*Cannabis sativa*; Cannabinaceae; geographic origin; chemotype; cannabinoïds; tetrahydrocannabinolic acid; tetrahydrocannabivarolic acid; environmental conditions.

Abstract—Culture in a phytotron of *Cannabis sativa* L. originating from S. Africa revealed the presence of two chemotypes varying in concentration of tetrahydrocannabinolic and tetrahydrocannabivarolic acids. We ascribe this to the genetic heterogenity of the seeds.

INTRODUCTION

Selon l'aire de répartition géographique du *Cannabis sativa* L., on a coutume de distinguer le Chanvre à résine de régions chaudes et sèches et le Chanvre à fibre des zones tempérées. Cette distinction se retrouve au niveau de la composition chimique des plantes: le tétrahydrocannabinol [1] et son précurseur l'acide tétrahydrocannabinolique caractérisant le Chanvre à résine tandis que les constituants majeurs du Chanvre textile sont le cannabidiol et l'acide cannabidiolique [2,3]. Chez le *Cannabis sativa* L. originaire d'Afrique du Sud, les cannabinoïdes principaux sont le tétrahydrocannabinol et son précurseur; on note l'absence de cannabidiol [4]. De plus, nous avons déjà indiqué l'importance des composés propyliques [5] homologues du tétrahydrocannabinol et de son acide [6] chez ce Chanvre.

Il nous a paru intéressant d'approfondir l'étude du Chanvre originaire d'Afrique de Sud. Dans ce but, nous avons entrepris de le cultiver dans les salles conditionnées du Phytotron du CNRS de Gif/Yvette selon les techniques déjà utilisées [7]. Nous écartons ainsi tout risque de fertilisation croisée et donc l'hybridation qui ne manque pas de se produire lors des cultures en plein champ de Chanvre de diverses origines [8,9]. Nous avons pu suivre, avec précision, les caractères morphologiques, physiologiques et chimiques des plantes cultivées, pendant trois générations, dans les deux conditions climatiques déjà retenues [7] à savoir: 32° le jour et 12° la nuit d'une part et 22° le jour et 12° la nuit d'autre part.