Natural Product Sciences
23(2) : 113-118 (2017)
https://doi.org/10.20307/nps.2017.23.2.113

Integracide K: A New Tetracyclic Triterpenoid from *Desmodium uncinatum* (Jacq.) DC. (Fabaceae)

Borice Tapondjou Tsafack[1], Beaudelaire Kemvoufo Ponou[1], Rémy Bertrand Teponno[1], Raymond Ngansop Nono[1], Kristina Jenett-Siems[2], Matthias F. Melzig[2], Hee Juhn Park[3,*], and Léon Azefack Tapondjou[1,*]

[1]*Department of Chemistry, Faculty of Science, University of Dschang, Box 67, Dschang, Cameroon*
[2]*Institut für Pharmazie (Pharmazeutische Biologie), Freie Universität Berlin, Königin-Luise-Str. 2-4, D-14195 Berlin, Germany*
[3]*Department of Pharmaceutical Engineering, Sangji University, 26339 Wonju, Korea*

Abstract – A new tetracyclic triterpenoid [4,4,24-trimethylcholesta-$\Delta^{8,9;14,15;24,28}$-trien-3β,11β,12α-triol-12-acetate, 3-sulfate] sodium salt (**1**), together with eight known compounds including ergosterol 5α,8α-endoperoxide **(2)**, 1,9-dihydroxy-3-methoxy-2-methylpterocarpan **(3)**, 3-*O*-β-*D*-2-acetyl-amino-2-deoxyglucopyranoxyloleanoic acid **(4)**, hydnocarpin **(5)**, derrone **(6)**, isovitexin **(7)**, erythrinin C **(8)**, and 5,4'-dihydroxy-2"-hydroxyisopropyldihydrofurano [4,5:7,8]-isoflavone **(9)**, were isolated from the EtOAc soluble fraction of the methanol extract of aerial part of *Desmodium uncinatum* collected in the western highland of Cameroon. The structures of these compounds were established by comprehensive interpretation of their spectral data mainly including 1D- (^{1}H and ^{13}C), 2D-NMR (^{1}H-^{1}H COSY, HMQC, HMBC) spectroscopic and ESI-TOF-MS mass spectrometric analysis. The isolation of an integracide-like compound from plant origin is a very unusual finding.
Keywords – *Desmodium uncinatum*, Fabaceae, Tetracyclic triterpenoid, Integracide, Isoflavonoids

Intoduction

The genus *Desmodium* is a large group of the Fabaceae family. It contains about 350 species which are mainly distributed in tropical and subtropical region of the world and about 28 species in China.[1] Most of its plants are herbs, shrubs or sub-shrubs but rarely trees. Besides their popularity as feeding stuffs, they are also used in traditional medicine.[2] Many species in this genus have a long history of medicinal uses and are recorded to have hepatoprotective effect.[3] *Desmodiun uncinatum* is a large perennial legume with stems that may grow several meters long and trail over surrounding vegetation. These cylindrical or angular stems are covered with short, hooked hairs that stick to hair or clothing. The roots of *D. uncinatum* have been found to stimulate germination of striga seeds and to inhibit radicle growth of the resulting seedlings; this combination represents the allelopathic mechanism associated with striga control.[4,5] Previous phytochemical investigations on these roots growing in Kenya have revealed the presence of isoflavanones including uncinanone A, B, C, D and E, and a pterocarpan uncinacarpan.[4,6] The present study was undertaken in order to characterize the chemical constituents of the aerial part of this plant growing in the western region of Cameroon and this report deals with the isolation and structural elucidation of nine compounds from the ethyl acetate soluble fraction of its methanol extract, amongst which one new tetracyclic triterpenoid derivative (integracide K) (**1**) and eight known compounds (**2** – **9**).

*Author for correspondence
Léon Azefack Tapondjou, Department of Chemistry, Faculty of Science, University of Dschang, Box 67, Dschang, Cameroon
Tel: +237-675004826; E-mail: tapondjou2001@yahoo.fr

Hee-Juhn Park, Department of Pharmaceutical Engineering, Sangji University, 26339 Wonju, Korea
Tel: +82-33-730-0564; E-mail: hjpark@sangji.ac.kr

Experimental

General procedure – The optical rotation was determined in MeOH with a MCP200 modular circular polarimeter. IR spectra were obtained on a Shimadzu FTIR-8400S spectrometer (Japan). ^{1}H NMR, ^{13}C NMR, COSY, HMQC and HMBC spectra were performed in deuterated solvents on a Bruker DRX-500 Spectrometer at 500 MHz/125 MHz and on a Bruker AVANCE 700 spectrometer (Bruker, Germany) at 700 MHz/175 MHz. All chemical shifts (δ) are given in ppm units with reference to tetramethylsilane (TMS) as internal standard and the coupling constants (*J*) are in Hz. ESI mass spectra

were carried out on an Agilent 6210 ESI-TOF mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Column chromatography was performed using 70 - 230 mesh and 230 - 400 mesh silica gel 60 (Merck), and sephadex LH-20. TLC was carried out on precoated silica gel 60 F_{254} (Merck) plates and spots were visualized by a UV lamp multiband UV-254/365 nm (Model UVGL-58 Upland CA 91786, U.S.A) and by spraying with 50% H_2SO4 and heating for 10 min at 110 °C.

Plant material – The aerial part of *D. uncinatum* was collected in the city of Dschang (Menoua Division, Western region of Cameroon) in August 2015. The plant identification was confirmed at the Cameroon National Herbarium, Yaound, where a voucher specimen was kept under the reference number HNC/5315.

Extraction and isolation – The powdered aerial part of *D. uncinatum* (4 kg) was extracted three times (each time for 24 h) with MeOH at room temperature followed by heating for 30 min before filtration. The combined filtrate was concentrated under reduced pressure to give the crude MeOH extract (168 g). Part of this extract (162 g) was suspended in distilled water (400 ml) and partitioned successively with ethyl acetate and *n*-butanol to yield after evaporation of solvents 78 g and 13 g of EtOAc and n-BuOH fractions, respectively. Part of the ethyl acetate fraction (73 g) was fractionated by a silica gel column chromatography using a gradient of ethyl acetate in *n*-hexane and thereafter a gradient of MeOH in ethyl acetate to give seven main fractions (A-G). Fraction E (3.5 g) was submitted to silica gel column chromatography eluting with hexane-EtOAc (1:1) to yield compound **5** (31 mg) and a mixture (sub-fraction E_1). Recrystallization of the sub-fraction E_1 in MeOH yielded compound **7** (26 mg). Fraction B (7 g) was purified over silica gel column chromatography eluted with an isocratic mixture of hexane-EtOAc (9:1) to afford compound **2** (15.6 mg) and a mixture (sub-fraction $B_{1)}$. Sub-fraction B_1 was chromatographed on a silica gel column using the mixture hexane-EtOAc (8.5:1.5) as an eluent to give compounds **3** (7.8 mg) and **6** (6 mg). Recrystallization of Fraction C (11 g) (eluted with hexane-EtOAc 40%) in EtOAc yielded compound **9** (9.5 mg) and the resulting filtrate was chromatographed on the silica gel column using the mixture hexane-EtOAc (6:4) as an eluent to afford compound **8** (46 mg). Fraction G (12 g) (eluted with EtOAc-MeOH (8:2)) was chromatographed on a silica gel column with EtOAc-MeOH (9.5:0.5) as an eluent to give seven sub-fractions (G1-G7). The sub-fraction G5 was subjected to silica gel column chromatography eluted with EtOAc-MeOH (9.5:0.5) to give compound **4** (12 mg) and a mixture (sub-fraction $B_{5\text{-}1)}$. The above mixture was purified by silica gel column chromatography eluted with EtOAc-MeOH (9.5:0.5) and thereafter by a sephadex LH-20 column chromatography eluted with MeOH to give compound **1** (25 mg).

Table 1. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) data of compound 1 (*J* in Hz)

Position	δ_H	δc
1	1.54 (2H, m)	34.2
2	2.18 (1H, m), 1.51 (1H, m)	33.3
3	3.62 (1H, d, *J* = 9.7; 4.2)	82.7
4		38.8
5	1.55 (1H, m)	50.9
6	1.69 (1H, m) 1.60 (1H, m)	18.4
7	2.32 (1H, m), 2.20 (1H, m)	26.8
8		125.5
9		140.3
10		36.7
11	4.10 (1H, brs)	67.3
12	4.96 (1H, d, *J* = 1.5)	77.5
13		46.8
14		147.3
15	5.50 (1H, brt)	120.3
16	2.31 (1H, m), 2.00 (1H, m)	36.2
17	1.85 (1H, m)	48.6
18	1.03 (3H, s)	24.0
19	0.99 (3H, s)	17.1
20	1.55 (1H, m)	33.6
21	0.84 (3H, d, *J* = 6.6)	18.3
22	1.51 (2H, m)	33.6
23	2.01 (1H,m), 1.88 (1H, m)	31.2
24		156.4
25	2.18 (1H, m)	34.9
26	0.98 (3H, d, *J* = 3.1)	22.4
27	1.00 (3H, d, *J* = 3.1)	22.3
28	4.71 (1H, brs) 4.66 (1H, brs)	107.8
29	0.74 (3H, s)	16.8
30	0.92 (3H, s)	28.7
31		170.4
32	1.96 (3H, s)	21.2
11-OH	5.31 (1H, d)	

The data were measured in DMSO with reference to TMS;

Integracide K – Amorphous yellow powder. $[\alpha]^{20}_D$ = –93.84 (c 0.065, MeOH); IR ν_{max} (NaCl) = 3400, 1643, 1226 cm^{-1}; ^{1}H and ^{13}C NMR: see Table 1; ESI-MS (positive mode): *m/z* 623.3024 $[M+Na]^+$, *m/z* 1223.6158 $[2M+Na]^+$, (calcd. for $C_{32}H_{49}O_7SNa_2$, 623.2994)

Ergosterol 5α,8α-endoperoxide (2) – white amorphous powder; ESIMS (positive mode): *m/z* 451.3263 $[M+Na]^+$ ($C_{28}H_{44}O_3Na$); ^{1}H-NMR (500 MHz, CD_3OD) : δ 0.87 (3H, s, H-18), 0.88 (6H, d, *J* = 6.5 Hz, H-26/H-27), 0.92 (3H, s, H-19), 0.94 (3H, d, *J* = 6.8 Hz, H-28), 1.00 (2H, m, H-15/H-16), 1.04 (3H, d, *J* = 6.6 Hz, H-21), 1.28 (1H, m, H-17), 1.38 (2H, m, H-11), 1.44 (2H, m, H-15/H-16), 1.46 (2H, m, H-1), 1.49 (1H, m, H-25), 1.54 (1H, m, H-2), 1.79 (1H, m, H-2), 1.80 (1H, m, H-14), 1.88 (1H, m, H-23), 1.95 (2H, m, H-4), 1.98 (2H, m, H-12), 2.08 (1H, m, H-20), 3.75 (1H, m, H-3), 5.20 (1H, d, *J* = 7.9 Hz, H-22), 5.25 (1H, d, *J* = 7.9 Hz, H-23), 6.28 (1H, d, *J* = 8.5 Hz, H-6), 6.56 (1H, d, *J* = 8.5 Hz, H-7); ^{13}C-NMR (125 MHz, CD_3OD): δ 12.1 (C-18), 16.6 (C-28), 18.1 (C-19), 19.5 (C-21), 19.9 (C-26), 20.2 (C-27), 21.1 (C-11), 23.6 (C-15), 29.0 (C-2), 33.3 (C-25), 34.6 (C-4), 35.1 (C-1), 37.4 (C-10), 39.0 (C-16), 39.5 (C-12), 40.1 (C-20), 43.0 (C-24), 44.7 (C-13), 51.8 (C-9), 52.0 (C-14), 56.3 (C-17), 65.5 (C-3), 79.3 (C-8), 82.0 (C-5), 131.0 (C-7), 132.3 (C-23), 136.0 (C-22), 136.1 (C-6).

1,9-Dihydroxy-3-methoxy-2-methylpterocarpan (3) – yellow amorphous powder; ESIMS (positive mode): *m/z*, 323.0920 $[M+Na]^+$($C_{17}H_{16}O_5Na$) ; ^{1}H-NMR (500 MHz, CD_3OD): δ 2.10 (3H, s, 2-CH_3), 3.40 (1H, m, H-6a), 3.47 (1H, m, H-6), 3.78 (3H, s, 3-OCH_3), 4.22 (1H, m, H-6), 5.64 (1H, d, *J* = 6.3 Hz, H-11a), 6.12 (1H, s, H-4), 6.31 (1H, d, *J* = 2.2 Hz, H-10) 6.34 (1H, dd, *J* = 8.0 and 2.2 Hz, H-8), 7.10 (1H, d, *J* = 8.0 Hz, H-7); ^{13}C-NMR (125 MHz, CD_3OD): δ 8.0 (CH_3-2), 38.2 (C-6a), 54.2 (3-OCH_3), 66.3 (C-6), 77.1 (C-11a), 91.2 (C-4), 98.6 (C-10), 101.3 (C-11b), 105.3 (C-2), 108.7 (C-8), 118.5 (C-6b), 125.4 (C-7), 155.2 (C-4a), 156.1 (C-1), 158.8 (C-9), 159.8 (C-3).

3-*O*-β-*D*-2-acetyl-amino-2-deoxyglucopyranoxyloleanoic acid (4) – white amorphous powder, ESIMS (positive mode): *m/z* 682.4247 $[M+Na]^+$ ($C_{38}H_{61}NO_8Na$); ^{1}H-NMR (500 MHz, CD_3OD) : δ 0.66 (3H, s, H-23), 0.72 (1H, m, H-5), 0.86 (3H, s, H-26), 0.88 (6H, s, H-29/H30), 0.90 (6H, s, H-26/H-24), 1.06 (1H, m, H-19), 1.10 (3H, s, H-27), 1.30 (2H, m, H-16), 1.46 (2H, m, H-1), 1.48 (1H, m, H-2), 1.49 (1H, m, H-11), 1.57 (2H, m, H-6), 1.57 (1H, m, H-19), 1.60 (2H, m, H-7/H-22), 1.75 (1H, m, H-7), 1.76 (3H, s, C$\underline{H}_3$CO), 1.78 (1H, m, H-22), 1.80 (1H, m, H-11), 1.82 (1H, m, H-2), 1.90 (1H, m, H-9), 2.30 (2H, m, H-17), 2.76 (1H, d, *J* = 10.0 and 2.8 Hz, H-18), 2.98 (1H, dd, *J* = 11.5 and 4.1 Hz, H-3), 3.04 (2H, m, H-4'/H-5'), 3.27 (1H, m, H-3'), 3.40 (1H, m, H-2'), 3.41 (1H, m, H-6'), 3.67 (1H, m, H-6'), 4.27 (1H, d, *J* = 8.3 Hz, H-1'), 5.18 (1H, brt, H-5), 7.70 (1H, d, *J* = 9.2 Hz, $\underline{H}$-NAc);^{13}C-NMR (125 MHz, CD_3OD): δ 15.5 (C-25), 16.7 (C-24), 17.6 (C-26), 18.3 (C-6), 22.6 (C-30), 23.0 (C-11), 23.2 (C$\underline{H}_3$CO), 23.4 (C-16), 26.2 (C-2), 26.3 (C-27), 27.6 (C-23), 28.1 (C-15), 30.8 (C-20), 32.7 (C-29), 32.9 (C-22), 33.3 (C-7), 36.7 (C-10), 33.8 (C-21), 38.4 (C-4), 38.8 (C-1), 39.4 (C-8), 41.7 (C-18), 41.8 (C-14), 45.7 (C-19), 45.8 (C-17), 47.5 (C-9), 55.4 (C-5), 56.4 (C-2'), 61.6 (C-6'), 71.5 (C-4'), 74.3 (C-3'), 77.2 (C-5'), 88.4 (C-3), 104.0 (C-1'), 122.0 (C-12), 144.4 (C-13), 169.2 ($CH_3\underline{C}$O), 179.3 (C-28).

Hydnocarpin (5) – yellow amorphous powder, ESIMS (positive mode): *m/z* 465.1244 $[M+Na]^+$ ($C_{25}H_{20}O_9Na$); ^{1}H-NMR (500 MHz, DMSO): δ 3.40 (1H, m, H_a of 3-CH_2OH), 3.59 (1H, m, H_b of 3-CH_2OH), 3.79 (3H, s, 3"-O$\underline{C}\underline{H}_3$), 4.28 (1H, m, H-2), 5.03 (1H, m, H-3), 6.20 (1H, d, *J* = 2.0 Hz, H-8'), 6.52 (1H, d, *J* = 2.0 Hz, H-6'), 6.82 (1H, d, *J* = 8.1 Hz, H-8), 6.88 (1H, dd, *J* = 8.1 and 1.8 Hz, H-7), 6.90 (1H, s, H-3'), 7.05 (1H, d, *J* = 1.8 Hz, H-3), 7.10 (1H, d, *J* = 8.5 Hz, H-5"), 7.62 (1H, dd, *J* = 8.5 and 2.2 Hz, H-6"), 7.68 (1H, d, *J* = 2.2 Hz, H-2"); ^{13}C-NMR (125 MHz, DMSO): δ 55.1 (3"-O$\underline{C}$H$_3$), 60.1 ($\underline{C}$H$_2$OH), 71.1 (C-2), 77.1 (C-3), 93.5 (C-8'), 98.5 (C-6'), 103.6 (C-3'), 103.7 (C-10'), 111.4 (C-5), 114.6 (C-2"), 115.3 (C-8), 117.2 (C-6"), 119.5 (C-5"), 120.6 (C-7), 123.8 (C-6), 124.6 (C-1"), 144.0 (C-10), 147.1 (C-9), 148.0 (C-4"), 148.3 (C-3"), 157.6 (C-9'), 162.1 (C-5'), 163.2 (C-7'), 163.3 (C-2'), 182.1 (C-4')

Derrone (6) – yellow amorphous powder, ESIMS (positive mode): *m/z* 337.1101 $[M+H]^+$ ($C_{20}H_{18}O_6$), $[M+Na]^+$ 359.0920; ^{1}H-NMR (500 MHz, CD_3OD): δ 1.30 (6H, s, H-5"/H-6"), 5.72 (1H, d, *J* = 10.0 Hz, H-3"), 6.25 (1H, s, H-6), 6.75 (1H, d, *J* = 10.0 Hz, H-4"), 6.80 (2H, d, *J* = 8.7Hz, H-3'/H-5'), 7.40 (2H, d, *J* = 8.7 Hz, H-2'/H-6'); ^{13}C-NMR (125 MHz, CD_3OD): δ 26.7 (C-5"/C-6"), 77.8 (C-2"), 99.3 (C-6), 101.4 (C-8), 113.0 (C-4"), 114.8 (C-3'/C-5'), 121.5 (C-1'), 124.6 (C-3), 127.3 (C-3"), 130.1 (C-2'/C-6'), 152.6 (C-2), 153.4 (C-8a), 156.1 (C-4'), 159.8 (C-7), 181.0 (C-4).

Isovitexin (7) – yellow amorphous powder; ^{1}H-NMR (500 MHz, CD_3OD): δ 3.37 (1H, m, H-4"), 3.49 (1H, m, H-3"), 3.78 (1H, m, H-5"), 3.91 (2H, m, H-6"), 4.19 (1H, m, H-2"), 4.92 (1H, d, *J* = 9.2 Hz, H-1"), 6.48 (1H, s, H-8), 6.57 (1H, s, H-3), 6.92 (2H, d, *J* = 8.7 Hz, H-3'/H-5'), 7.81 (2H, d, *J* = 8.7 Hz, H-2'/H-6'); ^{13}C-NMR (125 MHz, CD_3OD): δ 61.4 (C-6"), 70.4 (C-4"), 71.1 (C-2"), 73.9 (C-1"), 78.7 (C-3"), 81.20 (C-5"), 93.9 (C-8), 102.3 (C-3), 103.7 (C-10), 107.7 (C-6), 115.6 (C-3'/C-5'), 121.6 (C-1'), 128.0 (C-2'/C-6'), 157.2 (C-9), 160.5 (C-5), 161.3 (C-4'), 163.4 (C-7), 164.7 (C-2), 182.5 (C-4).

Erythrinin C (8) – yellow amorphous powder; ESIMS (positive mode): *m/z* 355.1221 $[M+H]^+$ ($C_{20}H_{18}O_6$), 377.1041

$[M+Na]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ 1.23 (3H, s, H-5"), 1.29 (3H, s, H-4"), 3.14 (2H, dd, *J* = 2.5 and 8.7 Hz, H-1"), 4.78 (1H, t, *J* = 8.7 Hz), 6.40 (1H, s, H-8), 6.84 (2H, d, *J* = 9.1, H-3'/H-5'), 7.37 (2H, d, *J* = 9.1, H-2'/H-6'), 8.07 (1H, s); ^{13}C-NMR (125 MHz, CD_3OD): δ 24.0 (C-5"/C-4"), 26.0 (C-1"), 71.0 (C-3"), 88.1 (C-8), 91.7 (C-2"), 105.8 (C-4a), 109.2 (C-6), 114.9 (C-3'/C-5'), 121.7 (C-1'), 123.2 (C-3), 130.3 (C-2'/C-6'), 153.5 (C-2), 157.6 (C-5), 157.5 (C-4'), 158.7 (C-8a), 166.4 (C-7).

5,4'-Dihydroxy-2"-hydroxyisopropyldihydrofurano [4,5:7,8]-isoflavone (9) – yellow amorphous powder, ESIMS (positive mode): *m/z* 355 $[M+H]^+$ ($C_{20}H_{19}O_6$), $[M+Na]^+$ 377; ^{1}H-NMR (500 MHz, DMSO): δ 1.15 (3H, s, H-5"), 1.17 (3H, s, H-4"), 3.20 (2H, dd, *J* = 8.5 and 5.3 Hz, H-1"), 4.80 (1H, t, *J* = 8.6 Hz, H-2"), 6.31 (1H, s, H-6), 6.83 (2H, d, *J* = 8.5 Hz, H-3'/H-5'), 7.37 (2H, d, *J* = 8.5 Hz, H-2'/H-6'), 8.35 (1H, s, H-2), 9.64 (1H, s, OH-9) 13.20 (1H, s, OH-5); ^{13}C-NMR (125 MHz, DMSO): δ 25.2 (C-5"), 26.1 (C-4"), 26.5 (C-1"), 70.6 (C-3"), 91.7 (C-2"), 94.0 (C-6), 104.0 (C-8), 105.3 (C-4a), 115.5 (C-3'/C-5'), 121.6 (C-1'), 123.0 (C-3), 130.7 (C-2'/C-6'), 152.5 (C-8a), 154.8 (C-2'), 157.9 (C-4'), 162.8 (C-5), 167.8 (C-7).

Results and Discussion

The structures of compounds **2** - **7** (Fig. 1) were determined by comparing their NMR (^{1}H, ^{13}C) and MS spectral data with those found of the literatures of ergosterol 5α,8α-endoperoxide (**2**),[7] 1,9-dihydroxy-3-methoxy-2-methylpterocarpan (**3**),[5] 3-*O*-*β*-*D*-2-acetyl-amino-2-deoxy-glucopyranoxyloleanoic acid (**4**),[8] hydnocarpin (**5**),[9] derrone (**6**),[10] isovitexin (**7**),[11] erythrinin C (**8**),[12] and 5,4'-dihydroxy-2"-hydroxyisopropyldihydrofurano[4,5:7,8]-isoflavone (**9**).[13]

Compound **1** obtained as yellow powder was positively reacted with Liebermann-Burchard reagent, indicating that it is a triterpernoid. Its positive ESIMS displayed two important pseudo-molecular ion peaks at *m/z* 623.3024 $[M+Na]^+$ ($[C_{32}H_{49}O_7SNa+Na]^+$) and *m/z* 1223.6158 $[2M+Na]^+$ ($[2C_{32}H_{49}O_7SNa+Na]^+$), thus indicating a molecular weight of 600 a.m.u and the presence of a sulfate group (SO_4Na). The presence of this function was confirmed by the characteristic vibration band in the IR spectrum at 1226 cm^{-1}.[14] When the ^{1}H NMR spectra of **1** were both recorded in DMSO-d_6 and in pyridine-d_5, the spectra obtained in DMSO-d_6 was quite indicative as it revealed the signal of only one free OH group. This fact was quite determinant for the elucidation of the structure. The ^{1}H, ^{13}C and HMQC NMR spectra of **1** showed eight methyl groups at δ_H 0.98 (CH_3-26)/δc 22.4 (d, *J* = 3.1), δ_H 1.00 (CH_3-27)/δ_C 22.3 (d, *J* = 3.1); δ_H 0.84 (CH_3-21)/ δ_C 18.3; δ_H 0.99 (CH_3-19)/δ_C 17.1; δ_H 1.03 (CH_3-18)/δ_C 24.1; δ_H

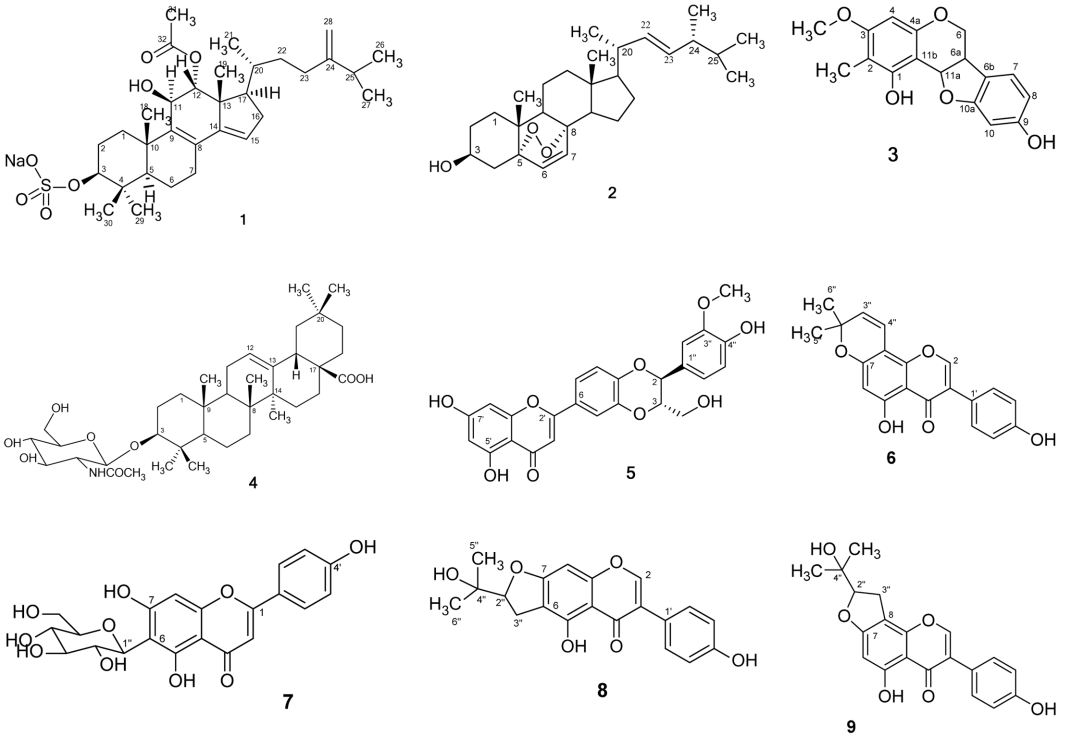

Fig. 1. Chemical structures of compounds isolated from *Desmodium uncinatum.*

0.74 (CH_3-29)/δ_C 16.9; δ_H 0.92 (CH_3-30)/δ_C 28.7, seven methylenes, seven methine groups (three of which are oxygenated), and eight quaternary carbons including one carbonyl and six olefinic carbons (three of them are tri-substituted olefinic double bonds conjugated at δ_C 140.3 (C-9); δc 125.5 (C-8) and δc 147.3 (C-14)). These NMR data were quite similar to those of integracide-like compounds;[15,16,17] therefore the dienic system was positioned at C-14–C-15 and C-8–C-9 based on the literature. This was thereafter confirmed by HMBC correlations of CH_3-19 (δ_H 0.99) to C-14, C-12 (δ_C 77.5), C-17 (δ_C 49.1) and C-13 (δ_C 46.8); H-12 (δ_H 4.96) to C-14 and C-9; OH-11 (δ_H 5.31) to C-9; CH_3-18 (δ_H 1.03) to C-9, C-1 (δ_C 34.2) and C-10 (δ_C 36.7). The position of this dienic system was also justified by the ^{1}H-^{1}H COSY cross peaks of H-15 (δ_H 5.50) to the methylene protons at δ_H 2.31 (m H_a-16) and 2.00 (m, H_b-16). Furthermore, the presence of signals at δ_H 4.71 (brs, H_a-28) and δ_H 4.66 (brs, H_b-28)/δ_C 107.8 (C-28) and δ_C 156.4 (C-24) indicate the presence of an exomethylene group. The position of this group was confirmed by HMBC cross peaks of CH_3-26 (δ_H 0.98) and CH_3-27 (δ_H 1.00) to C-24 (Fig. 2). The HMBC spectrum also showed cross peaks from CH_3-29 (δ_H 0.92) and CH_3-30 (δ_H 0.74) to C-3 (δ_C 82.7), C-4 (δ_C 38.8) and C-5 (δ_C 50.9) establishing the location of the gem dimethyl group at C-4 (δ_C 38.8). Other important cross peaks such as CH_3-21 (δ_H 0.84) to C-20 (δ_C 33.6), C-17 (δ_C 49.1) and C-22 (δ_C 33.6) indicated the presence of a methyl group at C-20. The presence of an isopropyl group in **1** was characterized by the signals at δ_H 0.98 (d, J = 3.1 Hz, CH_3-26)/δ_C 22.4, δ_H 1.00 (d, J = 3.1Hz, CH_3-27)/δ_C 22.3 and δ_H 2.18 (m, H-25)/δ_C 34.9 and confirmed by the ^{1}H-^{1}H COSY correlation of H-26 and H-27 with H-25. The connectivity of the isopropyl fragment at C-24 was confirmed by the HMBC correlation of CH_3-26, CH_3-27 to C-25 (δ_C 34.9) and C-24 (δ_C 156.4). The signal at δ_H 4.96 (d, J = 1.5 Hz, H-12)/δ_C 77.5 (C-12); δ_H 4.10 (brs H-11)/δ_C 67.3 (C-11) and δ_H 3.62 (dd, J = 9.7, 4.3 Hz, H-3)/δ_C 82.7 (C-3) indicated the presence of three oxygenated methine groups, they were located at C-12, C-11 and C-3, respectively, based on the observed ^{1}H-^{1}H COSY correlations of H-11 to H-12 and OH-11 (δ_H 5.31) and HMBC correlation of CH_3-29 and CH_3-30 to C-3. The ^{1}H NMR spectrum of **1** showed one singlet methyl signal at δ_H 1.96 (CH_3-32) correlated to the carbon signal resonating at δc 21.2 (C-32) in the HMQC spectrum. In the HMBC spectrum, this methyl group shows a cross peak with the carbonyl carbon at δc 170.4 (C-31), indicating the presence of one acetoxy group in **1**. Moreover, the presence of only one singlet signal at δ_H 5.31 in the ^{1}H NMR spectrum and

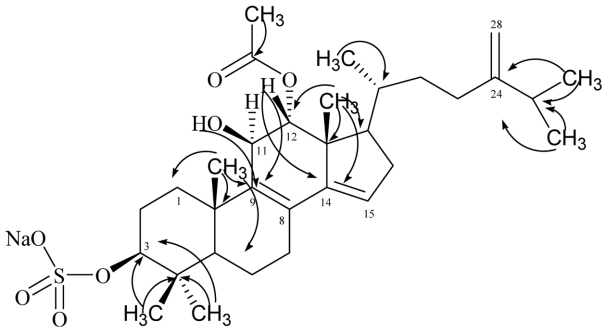

Fig. 2. Selected HMBC correlations of Integracide K (**1**).

assigned to OH-11 group indicated the substitution of other two oxymethines located at C-3 and C-12. As such, the acetoxy group was located at C-12 based on the literature comparison of the NMR data of **1** with those of integracide A-J,[15,16,17] thus the sulphate group was located at C-3. The stereochemistry of the different chiral centers were established based on the comparison of their spectral data with those of reported compounds. More precisely, the stereochemistry at C-11, C-12 and C-17 were confirmed based on the coupling constants observed for their corresponding proton atoms (Table 1) by comparing with those of reported integracides.[15,16,17] Thus the structure of compound **1** was elucidated as 4,4,24-trimethylcholesta-$\Delta^{8,9;14,15;24,28}$-trien-3β,11β,12α-triol-12-acetate,3-sulfate sodium salt.

Integracides are oxygenated tetracyclic 4,4-dimethylergostane terpenoids possessing a 12-acetyl-$\Delta^{8,14}$dien-11-ol moiety. Up to date, they have been reported only from fungi of *Fusarium* species such as *Fusarium sporotrichioides* 921,[18] *Fusarium compactum*[13] and *Fusarium sp*[14,15,16] and therefore seem to be the chemotaxonomic markers of these species. The present work is then the first report of an integracide-like compound from plants origin and so far from *Desmodium* species. Integracides have been found to exhibit important inhibitory activity against the HIV-1 integrase[15,19] and to display potent cytotoxic and anti-leishmanial activities.[16,17] Therefore, various bioactivities of *D. uncinatum* used in Cameroonian folk medicine might not only be attributed to its isoflavonoid but also to integracide K.

Acknowledgements

The authors are grateful to the Alexander von Humboldt Foundation (AvH), Bonn, Germany for the financial support of this work.

References

(1) Liguo, F.; Tanqing, C.; Kaiyong, L.; Hong, T.; Lin, Qi. Higher plants of China; Qingdao Publishing House: Qingdao, **2008**, p 152.

(2) Ma, X.; Zheng, C.; Hu, C.; Rahman, K.; Qin, L. *J. Ethnopharmacol*. **2011**, *138*, 314-332.

(3) Vedpal, Dhanabal, S. P.; Dhamodaran, P.; Chaitnya, M. V. N. L.; Duraiswamy, B.; Jayaram, U.; Srivastava, N. *J. Chem. Pharm. Res*. **2016**, *8*, 91-97.

(4) Tsanuo, M. K.; Hassanali, A.; Hooper, A. M.; Khan, Z.; Kaberia, F.; Pickett, J. A.; Wadhams, L. J. *Phytochemistry* **2003**, *64*, 265-273.

(5) Khan, Z. R.; Hassanali, A.; Overholt, W.; Khamis,T. M.; Hooper, A. M.; Pickett, J. A.; Wadhams, L. J.; Woodcock; C. M. *J. Chem. Ecol.* **2002**, *28*, 1871-1885.

(6) Guchu, S. M.; Yenesew, A.; Tsanuo, M. K.; Gikonyo, N. K.; Pickett, J. A.; Hooper, A. M.; Hassanali, A. *Phytochemistry* **2007**, *68*, 646-651.

(7) Correa, E.; Quiñones, W.; Torres, F.; Cardona, D.; Franco, A. E.; Robledo, S.; Echeverri, F. *Actual. Biol.* **2005**, *27*, 39-42.

(8) Wang, P.; Wang, J.; Guo, T.; Li, Y. *Carbohydr. Res.* **2010**, *345*, 607-620.

(9) Afifi, M. S. A.; Ahmed, M. M.; Pezzuto, J. M.; Kinghorn, A. D. *Phytochemistry* **1993**, *34*, 839-841.

(10) Máximoa, P.; Lourenço, A.; Savluchinske Feio, S.; Roseiro, J. C. *Z. Naturforsch*. C **2002**, *57*, 609-613.

(11) Peng, J.; Fan, G.; Hong, Z.; Chai, Y.; Wu, Y. *J. Chromatogr. A* **2005**, *1074*, 111-115.

(12) Tahara, S.; Ingham, J. L.; Mizutani, J. *Agric. Biol. Chem*. **1985**, *49*, 1775-1783.

(13) Dai, J.; Shen, D.; Yoshida, W.Y.; Parrish, S. M.; Williams, P. G. *Planta Med*. **2012**, *78*, 1357-1362.

(14) Brill, G. M.; Kati, W. M.; Montgomery, D.; Karwowski, J. P.; Humphrey, P. E.; Jackson, M.; Clement, J. J.; Kadam, S.; Chen, R. H.; McAlpine, J. B. *J. Antibiot.* **1996**, *49*, 541-546.

(15) Singh, S. B.; Zink, D. L.; Dombrowski, A. W.; Polishook, J. D.; Ondeyka, J. G.; Hirshfield, J.; Felock, P.; Hazuda, D. J. *Bioorg. Med. Chem*. **2003**, *11*, 1577-1582.

(16) Ibrahim, S. R. M.; Mohamed, G. A.; Ross, S. A. *Phytochem. Lett*. **2016**, *15*, 125-130.

(17) Ibrahim, S. R. M.; Abdallah, H. M.; Mohamed, G. A.; Ross, S. A. *Fitoterapia* **2016**, *112*, 161-167.

(18) Chatterjee, S.; Sarkar A.; Dutta, P. C. *J. Chem. Soc., Perkin Trans.* **1979**, *1*, 2914-2919.

(19) Singh, S. B.; Ondeyka, J. G.; Schleif, W. A.; Felock, P.; Hazuda, D. J. *J. Nat. Prod*. **2003**, *66*, 1338-1344.

Received January 29, 2017
Revised February 28, 2017
Accepted March 7, 2017