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THE CONSTITUTION OF ICACINOL, A NEW DITERPENE WITH A PIMARANE SKELETON FROM *ICACINA CLAESSENSIS*

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Abstract—Icacinol was isolated from the roots of *Icacina claessensis* (*Icacinaceae*). By X-ray diffraction analysis it was established that this new diterpene with a pimarane skeleton, corresponds to structure **4** (Scheme 1); spectroscopic properties were also discussed.

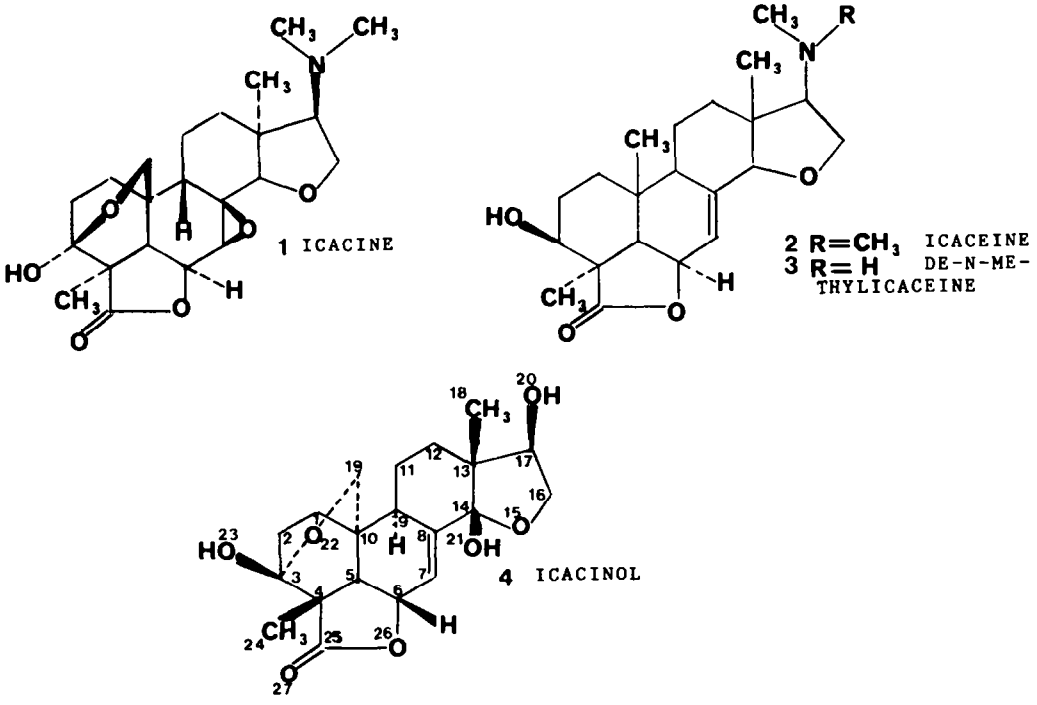
The root decoctions of *Icacina claessensis* and *guessfeldtii* are used in popular medicine (Zaïre) as anticonvulsant. From a search for the active principle(s) of *Icacina guessfeldtii*, the structures of three alkaloids, **1**, **2** and **3**, with a pimarane skeleton, were previously established.[1,2]

In continuation of our study on biologically active constituents of *Icacina claessensis*, a diterpene related to the same alkaloids skeleton was isolated. The present paper deals with the structure elucidation of this compound.

Isolation of icacinol **4**

The ethanolic extract of the roots was evaporated to dryness: the residue was suspended in 1% aqueous HCl and filtered. The filtrate was extracted several times with chloroform. The evaporated chloroform extract was treated with aceton; the aceton extract was further purified by prep hplc and precipitated in chloroform–ethanol mixture. Crystallization in acetonitrile–ethanol mixture afforded colourless crystals m.p. 250° (dec), $[\alpha]_D^{20} - 149°$.

Diterpenoids related to icacinol are doubtless the



Scheme 1.

Table 1. Atomic coordinates ($\times 10^4$) and B_{eq}(Å^2)

	x	y	z	Beq
C(1)	13166(7)	1399(0)	8280(4)	2.44
C(2)	13627(7)	1327(5)	9639(4)	2.52
C(3)	11770(6)	804(5)	10125(3)	2.08
C(4)	11318(6)	-401(5)	9639(3)	2.00
C(5)	11106(6)	-337(5)	8277(3)	1.99
C(6)	9132(7)	-1022(5)	7867(3)	2.34
C(7)	7901(7)	-658(5)	6734(4)	2.53
C(8)	8448(6)	219(5)	6133(3)	2.14
C(9)	10255(6)	1008(5)	6555(3)	2.15
C(10)	10981(6)	887(5)	7882(3)	1.99
C(11)	11945(7)	846(7)	5744(4)	2.76
C(12)	11022(7)	1100(6)	4485(4)	2.95
C(13)	9077(7)	390(5)	4001(3)	2.31
C(14)	7439(6)	409(5)	4886(3)	2.31
O(15)	6638(5)	1531(5)	4779(3)	3.04
C(16)	6645(9)	1913(6)	3598(4)	3.40
C(17)	7800(7)	1028(6)	2977(4)	2.61
C(18)	9719(10)	-792(6)	3712(4)	3.46
C(19)	9419(6)	1538(5)	8518(3)	2.23
O(20)	6261(6)	338(5)	2303(3)	3.49
O(21)	5834(6)	-378(6)	4590(3)	4.28
O(22)	9931(5)	1450(5)	9772(2)	2.54
O(23)	12009(6)	837(5)	11347(3)	3.02
C(24)	12919(7)	-1261(6)	10162(4)	2.66
C(25)	9100(7)	-733(5)	9847(4)	2.23
O(26)	7834(4)	-957(5)	8836(2)	2.66
O(27)	8429(4)	-826(5)	10770(2)	2.87
H(C1)	14224(92)	1068(45)	7902(44)	
H'(C1)	13428(81)	2160(50)	8064(46)	
H(C2)	14761(93)	879(47)	9865(46)	
H'(C2)	14006(81)	2086(52)	9993(43)	
H(C5)	12389(89)	-674(51)	8044(41)	
H(C6)	9482(73)	-1880(48)	7853(40)	
H(C7)	6680(78)	-1193(48)	6394(42)	
H(C9)	9902(84)	1768(49)	6439(42)	
H(C11)	12933(90)	1354(51)	5995(43)	
H'(C11)	12468(87)	187(55)	5824(46)	
H(C12)	10678(82)	1925(52)	4419(43)	
H'(C12)	11958(87)	940(45)	3971(44)	
H(C16)	5061(84)	1985(44)	3134(41)	
H'(C16)	7075(80)	2545(56)	3532(45)	
H(C17)	8560(81)	1295(48)	2479(46)	
H(C18)	8632(92)	-1246(51)	3515(45)	
H'(C18)	10349(81)	-1114(49)	4372(49)	
H"(C18)	10569(73)	-795(48)	3108(43)	
H(C19)	7907(88)	1242(46)	8352(41)	
H'(C19)	9635(79)	2265(57)	8402(46)	
H(O20)	6504(102)	-15(55)	1945(57)	
H(O21)	5426(78)	-378(44)	3677(48)	
H(O23)	13342(99)	806(51)	11555(45)	
H(C24)	14406(82)	-1040(44)	9993(43)	
H'(C24)	12677(80)	-2015(52)	9855(44)	
H"(C24)	12995(81)	-1273(47)	10978(47)	

precursors in the biogenesis of the lactonic alkaloids **1**, **2** and **3**.

X-ray analysis of icacinol **4**

Icacinol, $C_{20}H_{26}O_7$, crystallizes from acetonitrile–ethanol mixture as colourless monoclinic crystals, space group $P2_1$; a = 6.409(1), b = 11.931(3), c = 11.522(4)Å, β = 98.10(2)°; V = 872.2(4)Å^3; D_x = 1.44 g m^{-3} for Z = 2. 1220 independent reflections were measured on a Syntex $P2_1$ diffractometer using graphite monochromatized Cu$K\alpha$ radiation (λ = 1.54178 Å) to $2\theta_{max}$ = 113° and with the ω scan technique. 1190 of these were considered as observed ($I > 2\sigma(I)$) and included in the structure solution. An 18 atom fragment was found from a run of MULTAN 80.[3] This fragment, misplaced in the unit cell, could not be developed by Fourier methods. The TRADIR procedure of the programme DIRDIF[4] was used to determine a shift of 0.033 and 0.065 along x and z respectively. With these corrections, a straightforward run of DIRDIF revealed all the non-hydrogen atoms. The refinement was carried out with the SHELX 76 programme.[5] All the positions of hydrogen atoms were found on a Fourier difference map and refined with an overall isotropic temperature factor (2.92 Å^2). The final conventional R has the value of 0.040.

Table 1 gives the atomic coordinates following the atom numbering of Fig. 1. The absolute configuration is arbitrarily chosen. There are no unusual bond lengths or angles in the molecule. The endocyclic torsion angles are very similar to those observed on the icacine molecule.[1] Average difference 3.6°; largest difference 11° for C(7)–C(8)–C(9)–C(10) due to the replacement of the epoxy bridge by a double bond at C(7)–C(8).

Physical and chemical properties of icacinol **4**

After crystallization, icacinol is very slightly soluble in most organic solvents; it is moderately soluble in chloroform–ethanol mixtures, in pyridine and in acetonitrile. Icacinol affords by Se dehydrogenation a mixture of methylphenanthrenic and methylnaphthalenic compounds.[7] Upon acetylation at room

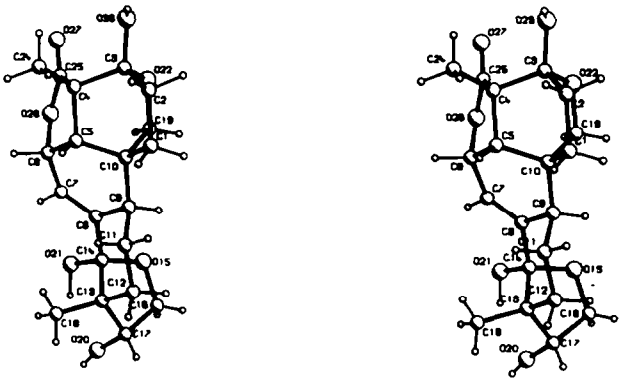


Fig. 1. Stereoscopic view of the molecule of icacinol and atom numbering (Programme PLUTO[6]).

temperature, it gives mainly a monoacetate together with a diacetate derivative and decomposition products. Molecular weight and elemental composition of icacinol monoacetate (420, $C_{22}H_{28}O_8$) and further of icacinol (378, $C_{20}H_{26}O_7$) were deduced from high resolution mass spectra measurements. IR spectrum of icacinol shows absorption at 3500 cm^{-1} (hydroxyl), 1740 cm^{-1} (lactone carbonyl) and 1675 cm^{-1} (double bond). The mass spectrum exhibits an ion at *m/z* 362 (6%) ($M^+ - 16$) and a fragmentation pattern (cf. experimental sections) which is closely similar to that of alkaloids **1**, **2** and **3**.[1,2] Interpretation of the ^1H NMR spectrum (on the basis of the X-ray results and previous data obtained from alkaloids **1**, **2** and **3**[1,2]) is given in the experimental section.

EXPERIMENTAL

Identity of Icacina claessensis

Specimen of the roots were collected around Kinshasa (Zaïre). Plants were identified by Dr. H. Breyne (Inera Herbarium). A voucher specimen has been deposited in the Inera Herbarium (National University of Zaïre, Kinshasa).

Chemical methods

The melting points (m.p.) were determined on a Mettler FP1/FP11 apparatus and the $[\alpha]_D$ value on a Perkin–Elmer 241 polarimeter. Preparative high performance liquid chromatography (prep HPLC) was performed using Merck silicagel Lichroprep Si 60 (particle size 5–20μ) dry-packed in a stainless column (22 mm i.d. × 25 cm length) connected to a Milton Roy pump (flow rate 12 ml/min), a Valco valve (sample loop, 1 ml) and a differential refractometer R401, Waters Associates. Analytical thin layer chromatography (TLC) was achieved using Merck DC-Fertighplatten Kieselgel $60F_{254}$ (20 × 10 cm, layer 0.25 mm); detection was performed by spraying 5%H_2SO_4 in ethanol and heating at 120° for 5 min: in these conditions, icacinol appeared as a bright yellow fluorescent spot under UV at 360 nm. IR spectra were obtained on a Perkin–Elmer 457 grating infrared instrument and UV spectra on a Perkin–Elmer 402 UV-V instrument. The ^1H NMR spectra were measured at 100 MHz on a Jeol FX 100 spectrometer; the data of the ^1H NMR spectra are reported as chemical shifts (interpretation, multiplicity, coupling constants J in Hz and number of protons). Mass spectra were obtained on a AEI MS 902 instrument; all peaks with an intensity > 10% relative to the base peak are reported as *m/z* values/intensity (interpretation and in cases of high resolution measurements, found and calculated *m/z* values).

Isolation of icacinol **4**

Air dried powder (500 g) of *Icacina claessensis* were lixiviated with 5 L ethanol. Evaporation of the ethanol solution afforded 110 g of a crude residue which was suspended in 200 ml 1% aqueous HCl. The suspension was extracted with $CHCl_3$ (10 times). The evaporation to dryness of the combined chloroform extracts afforded a white yellow residue (8 g). This residue was further extracted with acetone and the acetone solution purified by prep HPLC (mobile phase: $CHCl_3$–C_2H_5OH 46 : 4).

Yield of icacinol: 0.3%. m.p. 250°; $[\alpha]_D^{20} - 149°$ (c = 0.35, $CHCl_3/C_2H_5OH$ 8 : 2) TLC ($CHCl_3/C_2H_5OH$ 46 : 4) R_f 0.5; IR(KBr): 3500 (OH), 3020 (C=C), 1740 (CO), 1675 (C=C); ^1H NMR (100 MHz, hexadeuterioacetone): 6.22(7-H, dd, J = 5.1, 1.8, 1H), 5.03(6-H, dd, J = 6.9, 5.0, 1H), 4.48(20-H, dd, J = 9.2, 4.1, 1H), 3.90(16-H and 17-H, 3H), 2.21 to 1.15 (10H), 1.42(24-Me, s, 3H), 1.01(18-Me, s, 3H); MS (70 eV) 362/6 ($M^+ - 16$), 318/100, 290/28, 245/60, 227/16, 199/28, 195/14, 187/35, 173/37, 167/27, 159/33, 152/23, 145/43, 129/35, 117/37, 105/38, 91/59, 77/34, 69/25, 65/20, 55/55, 41/40.

Acetylation of icacinol

To a solution of 50 mg icacinol in 1 ml pyridine was added 1 ml acetic anhydride. After 12 h at room temperature, the solvents were evaporated *in vacuo* and the residue was worked up by prep HPLC (mobile phase: $CHCl_3$–C_2H_5OH 46 : 4) MS (70 eV) 420/1 (M^+ found 420.1764 calculated for $C_{22}H_{28}O_8$ 420.1783) 402/6 ($M^+ - H_2O$ found 402.1678 calculated for $C_{22}H_{26}O_7$ 402.1678).

Dehydrogenation of icacinol

Icacinol (10 mg) mixed with Se (50 mg) was heated at 300° for 10 h in a sealed capillary tube. The residue was taken up in n-hexane and purified by tlc (1 plate, n-C_6H_{12}); pertinent bands (strong absorption under UV) were eluted and UV spectra performed in n-hexane (typical UV λ_{max}, 346, 330, 316, 302, 281, 260, 254, 250 and 232 nm and 351, 335, 326, 320, 301, 289, 280 and 259 nm).

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