Tetrahedron Vol. 38, No. 3, pp. 359 to 362, 1982
Printed in Great Britain.

0040-4020/82/030359-04$03.00/0

IRIDOIDS IN EQUATORIAL AND TROPICAL FLORA—III†

ISOLATION AND PARTIAL SYNTHESIS OF 6-EPIAUCUBIN, A NEW GLUCOSIDIC IRIDOID

A. BIANCO* and P. PASSACANTILLI
Centro C.N.R. per lo studio della Chimica delle Sostanze Organiche Naturali and Istituto di Chimica Organica dell'Università di Roma, P. le Aldo Moro, 2-00185 Roma, Italy

M. NICOLETTI
Istituto dell'Orto Botanico di Roma, L. Cristina di Svezia, 24-00165 Roma, Italy

and

R. ALVES DE LIMA
Departamento de Quimica, Universidade Federal de Alagoas-57000 Maceiò, Brasil

(*Received in U.K. 25 June* 1981)

Abstract—The structure of a new glucosidic iridoid, 6-epiaucubin **1**, isolated from leaves of a tropical tree, *Tecoma chrysantha Jacq.*, was established by analysis of the spectroscopical data of **1** and its hexa-*O*-acetyl derivative **3**. Partial synthesis of **1** from its epimer, aucubin **2**, is also reported.

Tecoma genus belongs to the family of Bignoniaceae and is typical of tropical and subtropical areas; its wood is widely used for timbering and for export, whereas the roots and leaves particularly are used in traditional and local medicine to treat several diseases.[1] This genus has been chemically investigated mainly in its quinone and alkaloidic components, whereas few works have appeared on polar fraction.[2] *Tecoma chrysantha Jacq.* is a medium size tree, widely distributed in North and North-East Brazil, where it is known by the trivial name of "ipê amarelo".[3] As with other species of the same genus, we found alkaloidic components in the bark of the trunk; however, they are absent from the leaves, where we isolated 0.5–0.6% of a glucosidic fraction essentially consisting of three components. We report here the structure and the configuration of the most polar one, 6-epiaucubin **1**, and describe its partial synthesis from aucubin **2**.

RESULTS AND DISCUSSION

Compound **1** is a white amorphous powder with molecular formula $C_{15}H_{22}O_9$, $[\alpha]_D^{25} = -58.9$ and UV (MeOH): λ_{max} 204 nm (log ϵ 3.6), typical of the double bond of an unconjugated iridoid enol-ether system. On acid hydrolysis it gave glucose (1 mole) together with black products arising from the decomposition of the aglycone. The ^{1}H NMR spectrum‡ of **1** (D_2O) (see Table 1) confirms its iridoidic structure and indicates the absence of substitution at C(4) and C(5), by the shape of H-3 (dd, $J_{3,4} = 6.0$ Hz, $J_{3,5} = 1.5$ Hz). In the cyclopentane, ring a trisubstituted double bond is present, which may be located between C(7) and C(8) because of the broad signals of an olefinic proton (H-7, δ 5.84) and of a vinylic hydroxymethylene group (2H-10, δ 4.26). Furthermore, the spectrum exhibits doublets of two acetalic protons (H-1 and H-1'), a double doublet of the vinilic H-4, a broad signal at δ 4.70, which may arise from a proton geminal to a secondary allylic function, a complex signals pattern in the region between δ 2.5–3.0, attributable to two allylic protons (H-5 and H-9). The acetylation of **1** under mild conditions afforded the hexaacetate derivative **3**. In the IR spectrum of **3** no hydroxy group absorbances appeared. Therefore, in the aglycone moiety of **1**, two alcoholic functions must be present. The ^{1}H NMR spectrum of **3**, in comparison with **1**, showed acetylation paramagnetic shifts for the signals at δ 5.54 and 4.66, attesting previous trends for two alcoholic functions, secondary and primary, attributable to positions 6 and 10, respectively.

As expected, the ^{13}C NMR PND spectrum of **1** (Table 2) shows fifteen lines, where resonances due to the sugar moiety can be distinguished with high precision by comparison with reported data for other glucosidic iridoids.[4] Four olefinic carbon atoms were assigned on the basis of their chemical shift values and their multiplicities in SFORD spectra. In the same spectrum in CD_3OD, the doublet at 76.2 ppm and the triplet at 61.5 ppm confirm previous assignments for hydroxy functions, definitively excluding positions 5 and 9, whose carbon atoms resonate as doublets at the expected positions.

From the above data it is possible to assign to **1** the same structure as aucubin **2**. However, although the R_f values of these compounds are practically identical, as well as their chromatic reactions with vanillin and resorcin, physical (m.p., $[\alpha]_D$) and spectroscopic data (IR, ^{1}H and ^{13}C NMR) are different, thus indicating that **1** differs from **2** in the absolute configuration of one or more chiral centres. In fact, comparison of the ^{13}C NMR data of **1** and **2** (Table 2) shows indicative differences. As in other C(6) epimeric couples of iridoids,[4] the 6-α hydroxy group shows in comparison with the corresponding β epimer, typical shielding of C(6), C(5) and C(4), whereas C(1) and C(3) suffer sensible deshielding. These differences can be

†Part II. A. Bianco, M. Massa, J. U. Oguakwa and P. Passacantilli, *Phytochemistry* (1981) **20**, 1871 (1981).

‡NMR spectra of **1** were recorded either in D_2O and CD_3OD which are the most common solvents for glucosidic iridoids.

Table 1. ^{1}H NMR spectra assignments[a]

Compound (solvent)	1 D_2O	1 CD_3OD	2 D_2O	3 $CDCl_3$	8 $CDCl_3$	9 $CDCl_3$	10 $CDCl_3$	11 $CDCl_3$
H-1	4.98, d $J_{1,9}$=7.0	4.95	5.16, d $J_{1,9}$=5.0	4.7-5.3	5.15, d $J_{1,9}$=5.0	4.7-5.3	4.7-5.3	4.7-5.3
H-3	6.43, dd $J_{3,4}$=6.0 $J_{3,5}$=1.5	6.45	6.20, dd $J_{3,4}$=6.0 $J_{3,5}$=1.5	6.23, dd $J_{3,4}$=6.0 $J_{3,5}$=1.5	6.16, dd $J_{3,4}$=6.0 $J_{3,5}$=1.5	6.18, dd $J_{3,4}$=6.0 $J_{3,5}$=1.5	6.16, dd $J_{3,4}$=6.0 $J_{3,5}$=1.5	6.30, dd $J_{3,4}$=6.0 $J_{3,5}$=1.5
H-4	4.94, dd $J_{4,5}$=4.0	4.92	5.01, dd $J_{4,5}$=4.0	4.7-5.3	4.92, dd $J_{4,5}$=4.0	4.7-5.3	4.7-5.3	4.7-5.3
H-5	2.5-3.0	2.4-2.9	2.74, m	2.5-3.0	2.83, m	2.75, m	2,83, m	2.9-3.3
H-6	4.70, m[b]	4.70	4.46, m	5.54, m	5.29, m	4.46, m	5.32, m	-
H-7	5.84, bs	5.90	5.75, bs	5.74, bs	5.84, bs	5.84, bs	5.84, bs	6.15, bs
H-9	2.5-3.0	2.4-2.9	3.03, m	2.5-3.0	3.16, m	3.10, m	3.10, m	2.9-3.3
H-10	4.26, bs	4.15;4.40 J_{AB}=15.0	4.23, bs	4.66, bs	4.75, bs	4.75, bs	4.30, bs	4.7-5.3
H-1'	4.70, d[b] $J_{1',2'}$=7.0	4.65	4.70, d[b] $J_{1',2'}$=7.0	4.7-5.3	4.7-5.3	4.7-5.3	4.7-5.3	4.7-5.3

[a] Chemical shifts as δ ; coupling constants in Hz; d =doublet, dd =double doublet, m = multiplet, bs = broad signal.

[b] The shape of this signal was confirmed by thermal shift of HDO at 70° C.

Table 2. ^{13}C NMR spectra assignments[a]

Compound	1	1	2	2[b]	4[b]	5[b]	3	3	8	8
Solvent	D_2O	CD_3OD	D_2O	CD_3OD	CD_3OD	CD_3OD	$CDCl_3$	CD_3OD:$CDCl_3$	$CDCl_3$	CD_3OD:$CHCl_3$
C(1)	98.2	99.1	96.3	97.7	101,5	98.8	96.3	98.5	93.8	95.4
C(3)	142.4	143.2	140.4	141.5	155.6	154.0	141.1	143.3	139.7	140.9
C(4)	103.0	102.7	106.1	105.7	108.3	111.0	100.2	102.0	103.9	104.7
C(5)	40.0	41.9	43.3	46.1	42.6	47.0	37.9	40.3	39.3	40.6
C(6)	76.1	76.2	81.4	82.7	75.3	82.4	75.3	79.4	82.4	83.9
C(7)	128.8	129.6	129.4	130.3	129.9	129.9	126.8	128.6	127.2	128.1
C(8)	148.4	150.6	147.6	147.9	151.3	147.3	145.2	147.7	144.3	145.4
C(9)	47.1	47.7	47.2	47.9	45.7	45.9	46.2	48.1	46.8	47.8
C(10)	60.6	61.5	60.3	61.3	61.6	61.1	61.2	63.1	61.2	62.3
C(11)					170.9	172.1				
C(1')	99.3	99.9	99.2	99.9	100.4	100.2	96.3	99.2	95.9	97.1
C(2')	73.7	74.7	73.6	74.8	74.8	74.6	70.3	72.5	70.6	71.8
C(3')	76.9[c]	78.0[c]	77.0[c]	78.1[c]	78.2	78.1[c]	71.9[c]	74.1[c]	72.4[c]	73.5[c]
C(4')	70.4	71.4	70.4	71.4	71.5	71.3	67.9	70.0	68.3	69.4
C(5')	76.5[c]	77.6[c]	76.5[c]	77.8[c]	77.6	77.6[c]	71.4[c]	73.2[c]	71.9[c]	72.7[c]
C(6')	61.6	62.6	61.5	62.6	62.7	62.5	61.3	63.5	61.6	62.3
$OCOCH_3$							169.8	172.3	170.4	171.9
"							169.6	171.7	170.2	171.8
"							169.5	171.2	169.9	171.2
"							169.4	171.1	169.7	170.7
"							168.6		169.1	170.6
"									168.8	
$OCOCH_3$							20.6	21.9	21.1	21.3
"							20.4	21.6	20.9	20.9
"							20.2	21.5	20.6	20.8
"							20.1	19.3	20.4	

[a] Values in parts per million from Me_4Si; in CD_3OD: δ (Me_4Si) = δ (CD_3OD) + 49.6 ppm; in $CDCl_3$: δ ($CDCl_3$) + 77.0 ppm; in D_2O: CH_3OH was used as internal standard.

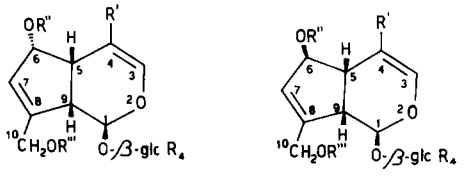
[b] Values reported in ref. 4, approximated to the first decimale figure.

[c] Values with same superscript in the vertical column are interchangeable.

also seen in Table 2 with the similar ones in the pair desacetyl asperulosidic acid 4/scandoside 5 (4-carboxylic derivatives of 1 and 2, respectively).[4] This is in agreement with ^1H NMR data of 1 and 2 and, in particular, with the chemical shift differences observed for the H-1 protons (δ 4.98 in 1, δ 5.16 in 2) and the coupling constants observed for $J_{1,9}$ (7.0 Hz in 1, 5.0 Hz in 2). On the basis of the considerations reported in a previous paper for epimeric iridoidic pairs,[5] a different configuration at C(6) centres of 1 and 2 can be hypothesized.

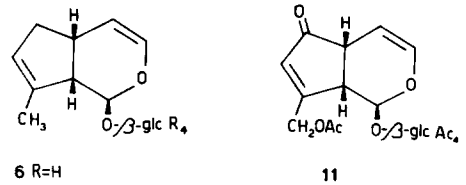
To verify the proposed structure, we performed the allylic hydrogenolysis[6] of 1 with lithium in liquid ammonia and obtained a bisdeoxy derivate 6, whose acetyl derivative 7 was identical to an authentic sample of tetra-*O*-acetyl-6,10-bisdeoxyaucubin (super-imposable IR and ^1H NMR spectra). This proved that the chiral centres C(1), C(5) and C(9) and the glucose moiety of 1 and 2 have the same absolute configurations. Therefore, the only possible difference between 1 and 2 must lie in the absolute configuration at C(6). To verify definitively this hypothesis we prepared 6-epiaucubin starting from aucubin.[7]

The acid hydrolysis of hexa-*O*-acetylaucubin 8 afforded the 6-deacetyl derivative 9, together with the 10-deacetyl derivative 10. Compound 9 was oxidized with Jones' reagent[8] affording the 6-oxo derivative 11, which, on reduction with $NaBH_4$, gave aucubin and 6-epiaucubin in about a 1:3 ratio. 6-Epiaucubin synthesised in this way was identical to 1 ($[\alpha]_D$, IR, ^1H and ^{13}C NMR) thus definitively proving structure 1.



1 R=R'=R''=R'''=H
3 R=R''=R'''=Ac R'=H
4 R=R''=R'''=H R'=CO_2H

2 R=R'=R''=R'''=H
8 R=R''=R'''=Ac R'=H
5 R=R''=R'''=H R'=CO_2H
9 R=R'''=Ac R'=R''=H
10 R=R'''=Ac R'=R''=H



6 R=H
7 R=Ac

11

As previously noted, epimerism at position 6 generates interesting effects in ^{13}C NMR. In particular, C(1) shows significant deshielding in α-OH vs. β-OH compounds, unexpected on the basis of electric field effects. Since in **1**, **2**, **4** and **5** the cyclopentane ring is rigidly held in a flat position, we propose that this effect could be due to different conformations of the dihydropyranic ring, between different hemichairs. This trend would be corroborated by the values of 1,9 coupling constants in glucosidic iridoids, also having saturated cyclopentane rings, varying in a large range, from a minimum value of *ca.* 2.5 Hz (*ca.* 110°) to 8.5 Hz (*ca.* 150°), followed in good accordance by corresponding changes of C(1) chemical shift values;[5] thus confirming the basic influence of C(6) stereochemistry on dihydropyranic ring conformations. ^{13}C NMR spectra of acetyl derivatives of **1**, **3**, and of **2** and **8**, were first recorded in deuteriochloroform, the usual solvent for these types of derivatives. However, comparison with data of **1** and **2** exhibits large solvent shift effects making difficult the determination of acylation sites. Direct use of deuteriomethanol was not possible because of poor solubility; the problem was solved using the minimum quantity of $CDCl_3$ to solubilize the product and subsequently adding larger quantity of CD_3OD. The values obtained this way for **3** and **8** are those expected and show clearly the effects of acetylation at positions 6 and 10; consequently this method can be generally useful to localize with precision the acylation sites.

To our knowledge, aucubin is the most common glucosidic iridoid, but so far it has been isolated only in plants of families peculiar to the temperate zone, in contrast to *Tecoma*, a genus typical of tropical areas. Furthermore, the finding of **2** is interesting being aucubin the only glucosidic iridoid which showed significant antibiotic activity in the aglycone form.[9] Thus pharmacological tests are now in progress to also prove the activity of 6-epiaucubin.

EXPERIMENTAL

PC: Schleicher & Scüll 2043 Mgl. Spray reagents: 2N H_2SO_4, vanillin (2 g vanillin, 4 ml conc. HCl, 100 ml MeOH), benzidine (0.5 g benzidine, 20 ml HOAc, 80 ml EtOH) and resorcin (5 g resorcin, 4 ml conc. H_2SO_4, 300 ml EtOH). Evaporation of volatile material was performed under reduced pressure. Natural abundance ^1H and ^{13}C NMR spectra were recorded on a Varian XL 100 Fourier transform NMR spectrometer.

Isolation of iridoids-containing fraction—*Tecoma chrysantha* Jacq. (Bignoniaceae) (Ipê amarelo) was collected in March 1980 near Fortaleza, Ceará (Brazil). Voucher specimens of the plant were identified and kept in the Universidade Federal de Alagoas-Maceiò (Brazil). Fresh aerial parts of the plant (0.6 kg) were triturated and extracted at room temperature with 90% EtOH (2 × 21.), until negative vanillin test. PC in BuOH–HOAc–H_2O (63:10:27) showed the presence of three iridoids (pink-lilac reaction with vanillin) having R_f values of 0.70 (unknown I), 0.66 (unknown II) and 0.27 (**1**). The ethanolic extract was concentrated to an aqueous suspension which was treated with decolourising charcoal (0.2 g). The resulting suspension was stratified on a gooch funnel (10 cm ⌀): monosaccharides were eluted with H_2O (6 l.), disaccharides with 5 and 10% EtOH (2 l. each), **1**, with 30% EtOH (3 l.), and the unknowns I and II with 50 and 80% EtOH (2.5 l. each).

6-Epiaucubin **1**. 30% EtOH fraction (2.45 g) was chromatographed on silica gel (100 g) in $CHCl_3$–MeOH (7:3) affording crude **1** (0.3 g) which was purified by a successive chromatography on silica gel in BuOH sat. H_2O: 141 mg of pure **1** was obtained as an amorphous powder. $[\alpha]_D^{25} = -58.9$ (MeOH, c 0.7), UV (H_2O), λ_{max} 204 nm (log ϵ: 3.6), IR (KBr), ν_{max} 3350, 2910, 1650, 1380, 1080, 1050, 1020 cm^{-1}; (Found: C, 51.96; H, 6.30. Calc. for $C_{15}H_{22}O_9$: C, 52.02; H, 6.40%).

Hexa-O-acetyl-6-epiaucubin **3**. **1** (150 mg) was treated with dry pyridine (1 ml) and Ac_2O (2 ml) for 1.5 h at room temperature. After addition of MeOH (5 ml), the solution was left for 20 min, then evaporated to give crude **3** (180 mg) which was chromatographed on silica gel in benzene–Et_2O (7:3) gave pure **3** as a colourless viscous oil. IR ($CHCl_3$): ν_{max} 2930, 1740, 1640, 1380, 1250, 1040 cm^{-1}.

Tetra-O-acetyl-6,10-bisdeoxyaucubin **7**. **1** (73 mg) was dissolved in liquid ammonia (50 ml) and abs. EtOH (1 ml), treated with Li (100 mg × 3) at −40° for 3 h, then left overnight at room temperature. The solid mixture was dissolved in H_2O, neutralized with bubbling CO_2 and finally extracted with EtOAc. The organic phase afforded crude **6** (40 mg) which was acetylated as previously described giving crude **7** (45 mg). By chromatography on silica gel in benzene–Et_2O (8:2) pure **7** was obtained (30 mg) which crystallised from EtOH as needles (m.p., mixed m.p., IR, ^1H NMR identical to those of an authentic sample of tetra-*O*-acetyl-6,10-bisdeoxyaucubin).

Penta-O-acetyl derivatives of **2**: **9** *and* **10**. Hexa-*O*-acetylaucubin **8** (2.0 g) was dissolved in dioxane–0.2N H_2SO_4 (1:1) (100 ml) and left for 24 hr at 40°. The solution was neutralized with pyridine, diluted with water and extracted with EtOAc. The residue obtained by evaporation of the organic phase was chromatographed on silica gel in benzene–EtOAc (8:2) to give unreacted **8** (1.3 g), **10** (264 mg) and **9** (290 mg). Compound **9**, colourless oil, $[\alpha]_D^{25} = -90.8$ (acetone, c 1.4). Compound **10**, colourless oil, $[\alpha]_D^{25} = -166.6$ (acetone, c 0.8).

Penta-O-acetyl-6-chetoaucubin **11**. Compound **9** (247 mg) was dissolved in acetone (10 ml) and carefully treated with Jones' reagent (3.5 ml) at 0–5° for 10 min. MeOH (10 ml) was added, the mixture was neutralized with pyridine, concentrated, diluted with water and extracted with EtOAc: crude **11** (300 mg) was obtained and purified by chromatography on silica gel in Et_2O–EtOAc (8:2). Compound **11** (107 mg) crystallised from EtOH as needles: m.p. 123.5–4.5°, UV (MeOH), λ_{max} 223 nm (log ϵ: 4.1).

Reduction of **11**: *6-epiaucubin* **1**. **11** (50 mg) was dissolved in EtOH 95° (10 ml) and treated with $NaBH_4$ for 10 min. After evaporation of the solvent, the residue was dissolved in water and treated with decolourising charcoal (500 mg, negative vanillin test of the solution). The suspension was stratified on a gooch funnel (1 cm ⌀), washed with water until negative salt test, then eluted with MeOH giving a residue (30 mg), which by chromatography on silica gel in BuOH sat. H_2O afforded **1** (15 mg) and **2** (5 mg) identified by comparison with authentic samples of aucubin and 6-epiaucubin (IR, ^1H and ^{13}C NMR).

Acknowledgement—This work was partially supported by CEME/CNPq (Brazil).

REFERENCES

[1]C. T. Rizzini and W. P. Mors, in *Botanica Economica Brasileira*, Ed. EPU and EDUSP, São Paolo, p. 166 (1976).
[2]R. Hegnauer, *Chemotaxonomie der pflanzen, III.* Birkhäuser Verlag, Basel and Stuttgard, p. 269 (1964).
[3]M. Pio Corrêa, *Dicionàrio das Plantas ûteis do Brasil e das exóticas cultivadas*, IV, Ed. Ministério da Agricultura, Rio de Janeiro, p. 318 (1952).
[4]R. K. Chaudhuri, F. U. Afifi-Yazar, O. Sticher and T. Winkler, *Tetrahedron* **36**, 2317 (1980).
[5]A. Bianco and P. Passacantilli, *Gazz. Chim. Ital.* **110**, 547 (1980).
[6]M. Smith, *Reduction* (Edited by L. Augustine), p. 98. Dekker, New York (1968).
[7]^1H NMR spectroscopical data of a synthetic 6-epiaucubin have been partially reported in A. Bianco, M. Guiso, C. Iavarone, P. Passacantilli and C. Trogolo, *Planta medica* **41**, 75 (1981).
[8]K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.* 39 (1946).
[9]O. Sticher, *New Natural Products and Plant Drugs with Pharmacological, Biological or Theurapeutical Activity* (Edited by H. Wagner and P. Wolff), p. 148. Springer-Verlag, Berlin (1977).