NEW IRIDOID GLUCOSIDES FROM *TECOMA CAPENSIS*[1]

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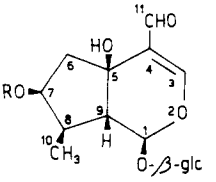
ABSTRACT.—*Tecoma capensis* contains, besides tecomoside (**1**), at least four new iridoids (**2, 3, 4,** and **5**) which resulted in 7-O-aryl-derivatives of **1**. The revised structure of tecomoside (**1**), previously proposed by ^{13}C-nmr data, has been demonstrated by chemical correlation of **1** with loganin (**7**).

Tecoma capensis Lindl. (Bignoniaceae) is a creeper, common to the tropical zone, which grows also in some temperate areas. In Italy, *T. capensis* is cultivated only in the Riviera ligure and in the South; very often it blooms, but rarely does it reach the size typical of its species. The examined sample was collected in Puerto de La Cruz (Tenerife, Canary Islands), in the natural habitat of this plant, and we isolated tecomoside (**1**), the first iridoid to be isolated containing an aldehydic function at C-4, which also has been isolated (2) from a sample of *T. capensis* cultivated in the Orto botanico dell'Università di Roma. In addition, Y. Hammouda *et al.* extracted from the leaves of *T. capensis* an amorphous glycoside (3), possibly related to an iridoid glycoside, esterified by the *p*MeO-cinnamic acid. In this paper, we describe the isolation and separation of four new iridoid glucosides, aryl-derivatives of **1**: the 7-*O*-(*p*-MeO)cinnamoyltecomoside (**2**), the 7-*O*-cinnamoyltecomoside (**3**), the 7-*O*-(*p*-OH)cinnamoyltecomoside (**4**), and the 7-*O*-(*p*-OH)benzoyltecomoside (**5**).

RESULTS AND DISCUSSION

The usual chromatographic purification of the extract of *T. capensis* afforded, besides tecomoside (**1**), two fractions (see Experimental): the first (fraction A) containing the nonseparable pair **2/3**, and the second (fraction B) containing the pair **4/5**. Analysis of these fractions by hplc allowed us to resolve the critical separation of **2** from **3** and of **4** from **5** (see figures 1 and 2) and revealed that these iridoids are accompanied by a great number of minor components that absorb in the uv in the same range and show a positive vanillin reaction identical to that of **1-5**, suggesting a probable iridoidic structure.



1 R=H
2 R=(*p*-OCH_3)cinnamoyl
3 R=cinnamoyl
4 R=(*p*-OH)cinnamoyl
5 R=(*p*-OH)benzoyl

The tecomoside-like structure (**1**) of the aglyconic part of **2, 3, 4,** and **5** was suggested by the ^{1}H-nmr spectra of these compounds (see table 1) which has been registered in methanol-d_4 owing to their low solubility in water-d_2. The comparison of ^{1}H-nmr spectra of **2, 3, 4,** and **5** with that of **1** (see table 1) shows a close relationship in the aglyconic signals, in chemical shifts, and in signal patterns; the only difference lies in the deshielding, in the spectra of **2, 3, 4,** and **5**, of a proton which is geminal to the secondary alcoholic function esterified by the aryl residue and, in the presence in the spectrum of **2**, of a signal pattern attributable to a *p*-OCH_3-*trans*-cinnamoyl residue; in the spectrum of **3**, of that relative to a *trans*-cinnamoyl residue; in the spectrum of **4**, of that

[1]Part VI in the series "Iridoids in Equatorial and Tropical Flora;" for part V see reference (1).

TABLE 1. ^{1}H-nmr data.[a]

Proton No.	Compounds				
	1	**2**	**3**	**4**	**5**
1	5.73 d $J_{1,9}$=1.5	5.84 d $J_{1,9}$=1.5	5.82 d $J_{1,9}$=1.5	5.82 d $J_{1,9}$=1.5	5.80 d $J_{1,9}$=1.5
3	7.30 s	7.36 s	7.33 s	7.33 s	7.33 s
6	2.8-2.0	2.8-2.2	2.8-2.2	2.8-2.2	2.8-2.2
7	3.80 m	5.08 m	5.08 m	5.08 m	5.00 m
8	1.73 m	1.95 m	1.95 m	1.95 m	1.95 m
9	2.8-2.0	2.8-2.2	2.8-2.2	2.8-2.2	2.8-2.2
10	1.10 d $J_{10,8}$=6.5	1.12 d $J_{10,8}$=6.5	1.10 d $J_{10,8}$=6.5	1.10 d $J_{10,8}$=6.5	1.02 d $J_{10,8}$=6.5
11	9.22 s	9.30 s	9.27 s	9.26 s	9.28 s
Hβ		6.40 d J=16.0	6.52 d J=16.0	6.30 J=16.0	
Hα		7.70 d J=16.0	7.72 d J=16.0	7.63 d J=16.0	
2″,6″		7.56[b] J=8.0	7.7-7.2	7.46[b] J=8.0	7.62[b] J=8.0
4″			7.7-7.2		
3″,5″		6.93[b] J=8.0	7.7-7.2	6.70[b] J=8.0	6.74[b] J=8.0
OCH_3		3.83 s			

[a]Chemical shift in ppm±0.01, coupling constant in Hz±0.5, using methanol-d_4 as solvent and TMS as internal standard.

[b]The AA′BB′ aromatic *p*-disubstituted system at 60 MHz appears as a simple AB system.

relative to a *p*-OH-*trans*-cinnamoyl residue; in the spectrum of **5**, of that relative to a *p*-OH-benzoyl residue.

The relationship suggested by ^{1}H-nmr data has been demonstrated, transforming **2, 3, 4,** and **5,** as well as **1,** in the common derivative **6**. The chemical relation between **1** and **2** was achieved by reducing the aldehydic function of **2** with $NaBH_4$ and hydrolyzing the aryl residue in alkaline medium: the obtained iridoid **6** was identical to that previously described (2), obtained by $NaBH_4$ reduction of **1**. In this hydrolysis, also, *p*-OCH_3-*trans*-cinnamic acid has been isolated and identified by comparison with an authentic sample (^{1}H-nmr spectra superimposable). The same reaction, performed on **3, 4,** and **5,** gave, together with **6,** also *trans*-cinnamic, *p*-OH-*trans*-cinnamic, and *p*-OH-benzoic acid, respectively, clearly indicating the aglyconic structure of these iridoids, as well as the structure of the aryl residue present. The acylation site has been identified by the comparison of ^{13}C-nmr data of **1** with those of **2, 3, 4,** and **5**. It is, in fact, well known that the esterification causes a characteristic downfield shift on the carbon bearing the hydroxyl function and upfield shifts on the carbons in the β position. In comparison with the ^{13}C-nmr spectrum of **1,** the spectra of **2, 3, 4,** and **5** (see table 2) appear, apart from the signals relative to the aryl residue, practically identical: the only difference lies in the deshielding of the signals relative to the C-7 (~3.5 ppm) and in the shielding of that signal relative to the C-8 (~1.2 ppm). These data prove that the hydroxyl function at C-7 is, in all the iridoids, the acylation site. It has not been possible to evidence the shielding of C-6 owing to the solvent signal pattern, which is superimposed on this signal.

Therefore, with the reported data, the structures of 7-*O*-(*p*-OCH_3)-*trans*-cinnamoyltecomoside, 7-*O*-*trans*-cinnamoyltecomoside, 7-*O*-(*p*-OH)-*trans*-cinnamoyltecomoside, and 7-*O*-(*p*-OH), benzoyltecomoside have been demonstrated for **2, 3, 4,**

TABLE 2. ^{13}C-nmr chemical shifts.[a]

Carbon No.	Compounds				
	1	**2**	**3**	**4**	**5**
1	97.1	96.4	96.4	96.4	96.4
3	162.7	162.6	162.6	162.6	162.7
4	126.9	126.5	126.5	126.7	127.5
5	72.1	71.6	71.6	71.6	71.6
6					
7	73.3	76.8	77.0	76.7	76.7
8	41.2	40.0	40.0	40.1	39.9
9	54.7	55.3	55.3	55.3	55.3
10	13.1	13.0	13.0	13.0	13.0
11	192.8	192.7	192.7	192.6	192.7
1′	100.1	100.3	100.3	100.2	100.3
2′	74.4	74.4	74.4	74.4	74.5
3′	78.5[b]	78.5[b]	78.5[b]	78.5[b]	78.6[b]
4′	71.6	71.6	71.6	71.6	71.6
5′	77.5[b]	77.5[b]	77.5[b]	77.6[b]	77.6[b]
6′	62.7	62.7	62.7	62.7	62.8
C=O		168.5	168.0	168.3	168.0
Cα		146.3	146.3	146.8	
Cβ		116.3	119.0	116.7	
1″		128.3	133.3	130.8	129.0
2″		131.0	130.1	131.2	133.7
3″		115.5	129.3	117.1	116.0
4″		162.6	131.5	162.6	162.7
5″		115.5	129.3	117.1	116.0
6″		131.0	130.1	131.2	133.7
OCH_3		55.9			

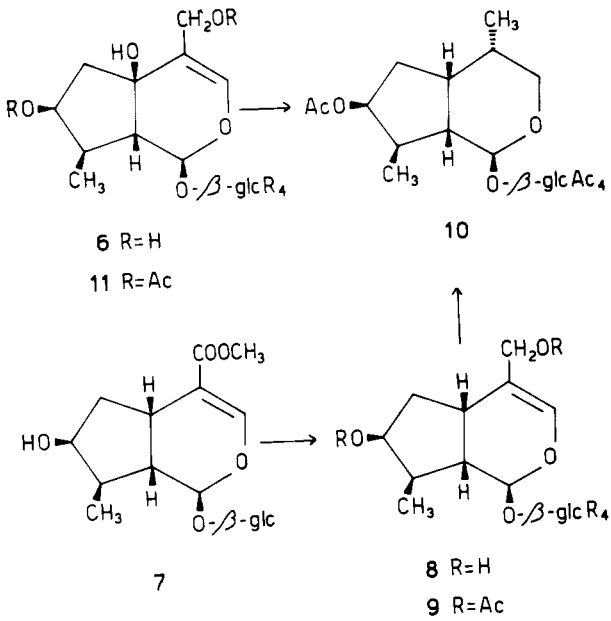
[a]Values in ppm ±0.1, using methanol-d_4 as solvent and TMS as internal standard.
[b]Assignments with the same superscript in the vertical column may be interchanged.

and **5**, respectively. Now we point out that the structure we report for tecomoside (**1**) is that recently revised (4,5) on the basis of a spectroscopic approach. In this paper we wish to add a chemical proof of the structure (**1**). Therefore, we decided to correlate **1** with loganin (**7**), which displays the substituent at C-7 and C-8, in an absolute configuration identical to that present in **1**. Loganin (**7**) has been reduced with $NaBH_4$ to the alcohol (**8**), successively transformed into the hexaacetate (**9**), which has been hydrogenolyzed with H_2/Pd-C. The hydrogenolysis follows a stereospecific route, giving only the compound **10**. The hydrogenolysis of hexaacetate (**11**), obtained by acetylation of **6**, afforded a hexahydroderivative, which in turn, resulted in a substance identical to **10**. In regard to the stereochemistry of this reduction, the hydrogenolysis of the hydroxyl at C-5 of **11** proves that the absorption of **11** on the catalyst occurred on the convex side of the molecule; in addition, in the cyclic iridoidic system the hydrogenolysis of OH-5 must occur with retention of configuration, and, therefore, the hydroxyl at C-5 of **11** must be in the same configuration as the H-5 of **10**. Thus, as regards the stereochemistry of the hydrogenolysis of **9**, the absorption on the catalyst occurred on the convex side of the molecule, which is obviously the more accessible one.

In conclusion, the described transformations chemically proved the absolute configuration of tecomoside (**1**) and supported the validity of the ^{13}C-nmr approach in the determination of the absolute configuration of chiral centers in iridoids (5-7).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—For column chromatography, silica gel, 70-230 mesh



(Merck) was used. For tlc, silica gel 60 F-254 (Merck) and cellulose (Merck) plates were used. For pc, Schleicher & Schüll no. 2043 Mgl paper was used. The spray reagents were 2N H_2SO_4, vanillin (vanillin 2 g, HCl 4 ml, methanol 100 ml), benzidine (benzidine 0.5 g, acetic acid 20 ml, ethanol 80 ml), resorcin (resorcin 5 g, H_2SO_4 4 ml, ethanol 300 ml) and 2,4 DNF (2,4 DNF 0.2% in 2N HCl).

The instruments used were: ^{1}H-nmr, Varian EM 360; ^{13}C-nmr, Varian CFT-20; ir, or, Perkin-Elmer 257 and 141; uv, Cary 219; hplc, Waters 6000 A equipped with a uv detector, Perkin-Elmer LC 55 B.

Evaporation of volatile materials was performed under reduced pressure. Elemental analysis of **2, 3, 4,** and **5** gave satisfactory results.

ISOLATION OF IRIDOIDIC FRACTION.—*Tecoma capensis* Lindl. (Bignoniaceae), which is also known as *Tecomaria capensis* (Thunb.) Spach, was collected in September 1980 in Puerto de La Cruz, Tenerife (Canary Islands) when it was in flower. Voucher specimens of the plant were identified by Dr. Anna Francesconi in the herbarium of the Istituto di Botanica dell'Università di Roma.

The fresh aerial parts of *T. capensis* (1 kg) were extracted with ethanol 95° at room temperature for three days. Pc in *n*-butanol-acetic acid-water (63:10:27) showed at least two spots with pink-lilac reaction with vanillin reagent and Rf 0.63 (**2, 3, 4,** and **5**) and 0.19 (**1**). The ethanolic extract was concentrated to an aqueous suspension, which was treated with decolorizing charcoal (200 g), and then the obtained suspension was stratified on a layer of silica gel in a gooch funnel (14 cm Ø). Monosaccharides were eluted with water (6 liters); di- and oligo-saccharides with 5% ethanol (1 liter) and 10% ethanol (1 liter); **1** and small quantities of **2, 3, 4,** and **5** with 30% (4 liters) and 50% (3 liters) ethanol (fraction 1); **2, 3, 4,** and **5** with 80% ethanol (10 liters) (fraction 2). Fraction 2 (4.5 g) was chromatographed on silica gel in chloroform-methanol (8:2) giving the nonseparable mixture of **2** and **3** (0.4 g, fraction A) and the nonseparable mixture of **4** and **5** (0.43 g, fraction B). Fraction 1 (5.5 g) was chromatographed on silica gel in $CHCl_3$-MeOH (75:25), giving 1.2 g of crude **1**, which was purified by a successive chromatography on silica gel in *n*-butanol-saturated with water; 0.8 g of pure **1** was obtained as white amorphous powder.

Fraction A (80 mg) was chromatographed on semipreparative hplc μ-Bondapak C_{18} column (30 cm x ¼ in, grain size 10 μ) eluting with methanol-water (1:1), flow rate 3.0 ml/min. Compounds **2** (40 mg) and **3** (24 mg) were obtained, both as white amorphous powders. Hplc chromatogram (see figure 1) showed the presence of small quantities of other unknown iridoid-type compounds.

Compounds **2** $[\alpha]^{25}D$ −63.8 (MeOH, *c* 0.1); uv (MeOH): λ max (log ε), 228(4.2), 310(4.4) nm; ir (KBr): ν max 3500, 1700, 1630, 1605, 1500, 1050 cm^{-1}.

Compound **3** $[\alpha]^{25}D$ −69.8 (MeOh, *c* 0.1); uv (MeOH): λ max (log ε), 217(4.1), 278(4.4) nm: ir (KBr): ν max 3500, 1705, 1640, 1570, 1500, 1450 cm^{-1}.

Fraction B (70 mg) was chromatographed on the above-described semipreparative hplc column, eluting with MeOH-H_2O (4:6), flow rate 3.0 ml/min. Compounds **4** (40 mg) and **5** (15 mg) were obtained as white amorphous powders. Also, in this chromatography (see figure 2) appeared small quantities of other unknown iridoid-type compounds.

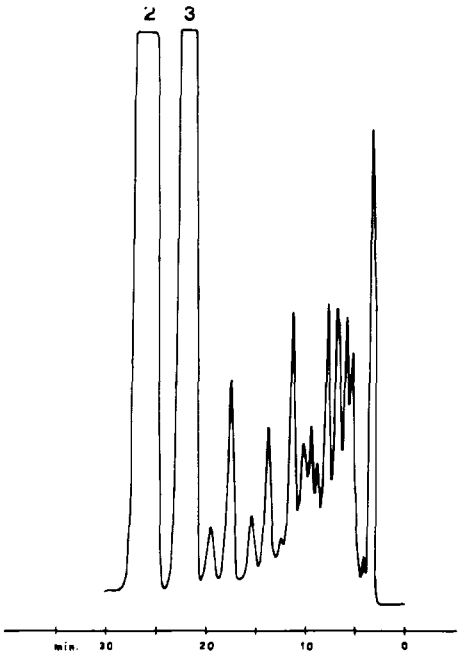


FIGURE 1. Semipreparative hplc of fraction A.

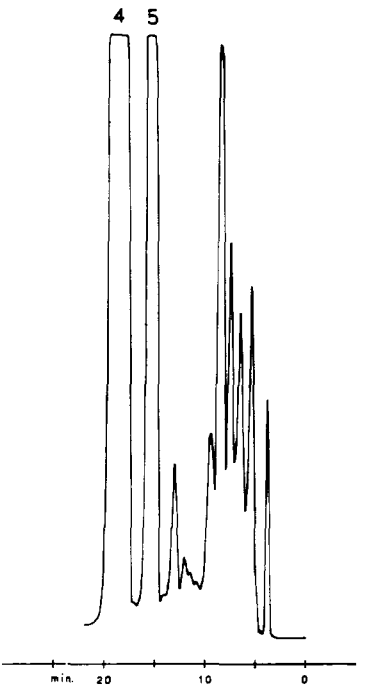


FIGURE 2. Semipreparative hplc of fraction B.

Compound **4** $[\alpha]^{25}D-64.9$ (MeOH, *c* 0.1); uv (MeOH): λ max (log ε) 226(4.2), 310(4.2) nm; ir (KBr): ν max 3400, 1720, 1690, 1630, 1605, 1590, 1510, 1440 cm^{-1}.

Compound **5** $[\alpha]^{25}D-64.7$ (MeOH, *c* 0.1); uv (MeOH): λ max (log ε) 260(4.3) nm: ir (KBr): ν max 3400, 1705, 1650 cm^{-1}.

REDUCTION OF **2, 3, 4,** AND **5**.—Compound **2** (100 mg) dissolved in water (10 ml) was treated with an excess of $NaBH_4$ (5 eq.) for 15 min at room temperature. 2N NaOH (10 ml) was added to solution and then left at room temperature overnight. The cooled solution was acidified with cold 2N HCl at ~ pH 3, extracted with ether, and immediately treated with decolorizing charcoal (1.5 g). The suspension was stratified on a gooch funnel (1 cm Ø), the charcoal was washed with water until pH 7 and negative salt test were obtained; then it was eluted with MeOH (200 ml), giving 60 mg of crude **6,** which was purified by chromatography on silica gel (5 g) in *n*-butanol saturated with water, affording 50 mg of pure **6** as hygroscopic amorphous powder. Compound **6** resulted identical to that obtained by reduction of tecomoside (**1**) (2) (ir and ^1H-nmr superimposable). The ether solution, dried on Na_2SO_4, afforded 20 mg of *p*-MeO-*trans*-cinnamic acid, identified by comparison with an authentic sample. The same procedure performed on **3, 4,** and **5** yielded **6,** together with *trans*-cinnamic acid, *p*-OH-*trans*-cinnamic acid, and *p*-OH-benzoic acid, respectively—all identified by comparison with authentic samples.

REDUCTION OF **7**.—Loganin (**7**) (150 mg) was treated with $NaBH_4$ for three days at room temperature in water (10 ml); every 12 h, $NaBH_4$, in molar ratio 2:1, was added. The solution was neutralized by bubbling carbon dioxide through it, charcoal (1.5 g) was added, and the resulting suspension was stratified on a gooch funnel (1 cm Ø).

The charcoal layer was washed with water until a negative salt test was obtained, then it was eluted with methanol (200 ml), affording 120 mg of crude **8,** which was purified by chromatography on silica gel in *n*-butanol saturated with water. This gave 100 mg of pure **8** as a colorless amorphous powder. ^1H-nmr (D_2O): δ 6.22 (H-3, bs) 5.31 (H-1, bs), 4.10 (2H-11, bs), 1.02 (3H-10, d, $J_{10,8}$=6.5 Hz).

HEXAACETYLDERIVATIVE **9**.—Compound **8** (100 mg), dissolved in dry pyridine (0.5 ml), was treated with Ac_2O (1.0 ml) for 2 h at room temperature. After adding methanol (5 ml), the solution was left for 15 min, then evaporated to give a residue (120 mg), which was chromatographed on silica gel (10 g) in *tert*-butyl-methyl-ether-benzene (2:8), yielding pure **9** (95 mg) as colorless viscous oil. ^1H-nmr ($CDCl_3$): δ 6.25 (H-3, bs), 1.00 (3H-10, d, $J_{10,8}$=6.5 Hz).

HYDROGENOLYSIS OF **9**.—Pd-C 10% (15 mg) was suspended in ethanol 95° (15 ml) and saturated with H_2. Then **9** (95 mg), dissolved in ethanol 95° (40 ml), was added and hydrogenated in a low pressure

apparatus for 45 min at room temperature. The catalyst was filtered off; the solution was evaporated, and the residue (91 mg), chromatographed on silica gel in benzene-ether (4:6), gave pure pentaacetyl-derivative **10** as a viscous colorless oil (75 mg); ^{1}H-nmr ($CDCl_3$); δ 1.08 (3H-10, d, $J_{10,8}$=6.5 Hz), 0.80 (3H-11, d, $J_{11,4}$=6.5 Hz).

HEXAACETYLDERIVATIVE **11**.—Compound **6** (100 mg) has been acetylated and worked up as described for **9**. Crude **11** was chromatographed on silica gel in *tert*-butyl-methyl-ether-benzene (4:6) affording pure **11** (90 mg) as colorless viscous oil; ^{1}H-nmr ($CDCl_3$): δ 6.30 (H-3, bs), 1.05 (3H-10, d, $J_{10,8}$=6.5 Hz).

HYDROGENOLYSIS OF **11**.—Compound **11** (90 mg) was treated with H_2/Pd-C and purified as described for **9**, giving a pure pentaacetyl-derivative (70 mg) identical to **10** (^{1}H-nmr superimposable).

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LITERATURE CITED

1. A. Bianco, P. Passacantilli, M. Nicoletti, and R. Alves De Lima, *Gazz. Chim. Ital.*, **112**, 227 (1982).
2. A. Bianco, M. Guiso, C. Iavarone, and C. Trogolo, *Gazz. Chim. Ital.*, **105**, 195 (1975).
3. Y. Hammouda and N., Khalafaliah, *Pharmazie*, **26**, 640 (1971).
4. A. Bianco, P. Caciola, M. Guiso, C. Iavarone, and C. Trogolo, *Gazz. Chim. Ital.*, **111**, 201 (1981).
5. S. Damtoft, S. R. Jensen, and B. J. Nielsen, *Phytochemistry*, **20**, 2717 (1981).
6. A. Bianco, P. Passacantilli, G. Polidori, and M. Nicoletti, *Org. Magn. Res.*, in press (1983).
7. A. Bianco, P. Passacantilli, and G. Polidori, *Planta Med.*, **46**, 38 (1982).