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COMBREGLUTININ, A HYDROLYZABLE TANNIN FROM *COMBRETUM GLUTINOSUM*

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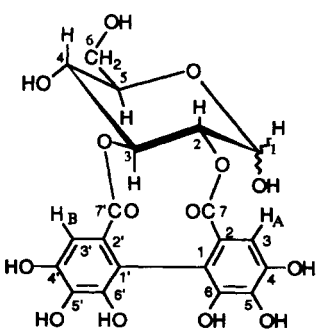
ABSTRACT.—Combreglutinin [**4**], a hydrolyzable tannin, was isolated from the leaves of *Combretum glutinosum*, and its macrocyclic structure was established on the basis of ^{1}H- and ^{13}C-nmr and 2D nmr spectroscopic data and by chemical methods. The three known tannins 2,3-(*S*)-hexahydroxydiphenoyl-D-glucose [**1**], punicalin [**2**], and punicalagin [**3**] were also isolated.

Leaves of *Combretum glutinosum* Perr. (Combretaceae) have traditionally been used as a diuretic and for the treatment of jaundice. We have previously reported that a MeOH extract of *C. glutinosum* inhibited hepatitis B virus surface antigen (1). However, the components responsible for this activity were not yet identified. We report here the isolation of four tannins from this extract, namely, 2,3-(*S*)-hexahydroxydiphenoyl-D-glucose [**1**], punicalin [**2**], punicalagin [**3**], and the new combreglutinin [**4**].

RESULTS AND DISCUSSION

Leaves of *C. glutinosum*, collected in Senegal, were extracted in a Soxhlet with CH_2Cl_2, then with MeOH. Chromatography of the MeOH extract on Sephadex LH-20 gave four products: 2,3-(*S*)-hexahydroxydiphenoyl-D-glucose [**1**] (0.1% yield of dry leaves), punicalin [**2**] (0.03%), punicalagin [**3**] (2%), and combreglutinin [**4**] (0.4%).

2,3-(*S*)-Hexahydroxydiphenoyl-D-glucose [**1**], previously isolated from *Eucalyptus delegatensis* (Myrtaceae) (2) and *Terminalia catappa* (Combretaceae) (3), and punicalin [**2**] and punicalagin [**3**], originally isolated from *Punica granatum* (Punicaceae) (4,5) and *T. catappa* (3), were identified from analysis of their ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY nmr spectral data and comparison with previously described data (5,6). Duplicate signals in the nmr spectra of **1–3** indicated the presence of α- and β- anomers of glucose (5,6). This epimeric equilibrium was due to the presence of a free hydroxyl group at the anomeric center. The ratio of α- and β-glucose for tannins **1–3** was estimated by integration of proton signals in their ^{1}H-nmr spectra as 60% and 40%, respectively. Identity of tannins **2** and **3** with punicalin and punicalagin, respectively, was further confirmed by hplc.



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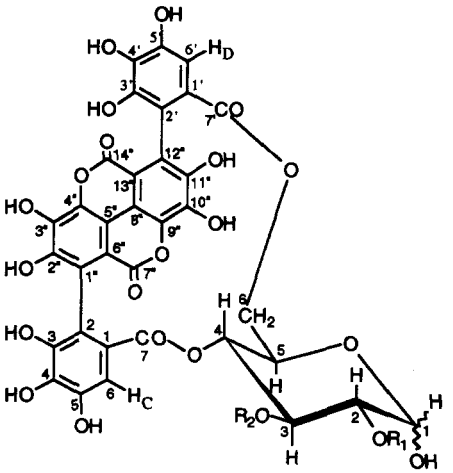
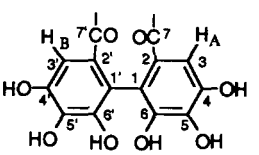
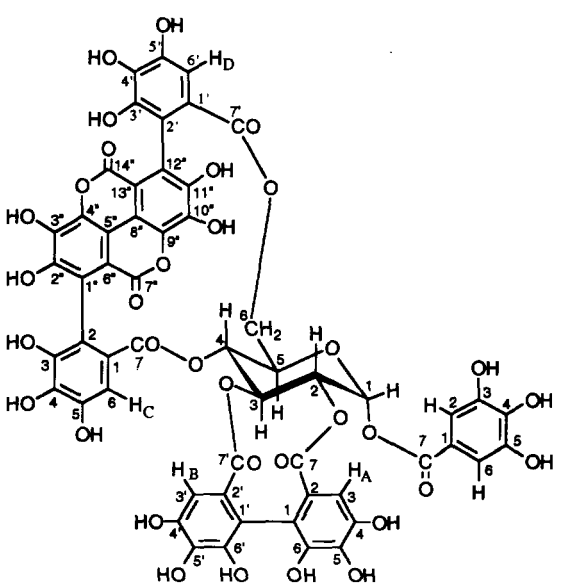


2 R_1,R_2=H

3 R_1,R_2=



Combreglutinin [**4**] was isolated as a brown amorphous powder with $[\alpha]_D^{20}$ −60° (MeOH). Its negative-ion fabms displayed a pseudomolecular $[M-H]^-$ anion at *m/z* 1235, and the hrfabms indicated a molecular formula of $C_{55}H_{32}O_{34}$. The ^{13}C-nmr spectrum of combreglutinin [**4**] showed only six carbon signals for the carbohydrate moiety in the δ 60–100 range. The observation of ^{1}H- and ^{13}C-nmr signals for one sugar anomer, together with the chemical shift at δ 6.32 of the anomeric proton (H-1) indicated that the anomeric hydroxyl was acylated. The presence of a glucopyranose ring with $^4C_1$ conformation (2,7) was deduced from the analysis of the proton coupling



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constant values of the carbohydrate protons: $J_{1,2}=3.5$ Hz, $J_{2,3}=J_{3,4}=J_{4,5}=J_{5,6}=10$ Hz and $J_{6,6}=11$ Hz. The value of the coupling constant for H-1 (3.5 Hz) and the ^{13}C-nmr chemical shift for C-1 (δ 89.7) indicated 1-α acylation of glucose. The deshielding by more than 1 ppm of the chemical shifts of the other protons in the glucopyranose moiety indicated all the hydroxyl groups to be acylated. A singlet of two protons at δ 7.15 suggested the presence of a galloyl group and four other aromatic protons were observed at higher field. The ^{13}C-nmr spectrum of **4** exhibited seven carbonyl carbon signals in the δ 157–170 region. The long-range ^{1}H-^{13}C-nmr shift-correlation spectrum of **4**, optimized for J_{CH} 7 Hz (Table 1) showed $^{3}J_{CH}$ cross-peaks between the carbonyl carbon atom at δ 164.7 and both the glucose proton H-1 at δ 6.32 and the two-proton singlet at δ 7.15 (galloyl H-2,6), indicating thus that the galloyl group acylated the *O*-1 of glucose.

TABLE 1. ^{1}H- (300.13 MHz), ^{13}C- (75.45 MHz) Nmr and Long-Range ^{1}H-^{13}C COSY Data for Combreglutinin [**4**] (Me_2CO-d_6).

	δ_C	δ_H (*J* Hz)	$^{2}J_{C\text{-}H}$ and $^{3}J_{C\text{-}H}$
Glucose			
C-1	89.7	6.32 (d 3.5)	
C-2	73.1	5.15 (dd 3.5, 10)	
C-3	75.6	5.38 (t 10)	
C-4	70.7	4.90 (t 10)	
C-5	70.8	3.38 (t 10)	
C-6	64.8	4.40 (dd 10, 11)	
		2.10 (d br 11)	
1α-Galloyl			
C-1	120.1		7.15
C-2, C-6	110.5	7.15 s	7.15
C-3, C-5	145.9		7.15
C-4	139.6		7.15
C-7	164.7		7.15; 6.32 (glc H-1)
2,3-Hexahydroxydiphenoyl			
C-1	114.3		6.40 (H_A)
C-2	126.1[a]		
C-3	107.1	6.40 s (H_A)	
C-4	145.1		6.40 (H_A)
C-5	136.2		6.40 (H_A)
C-6	144.3[c]		
C-7	168.1		6.40 (H_A); 5.15 (glc H-2)
C-1′	114.6		6.60 (H_B)
C-2′	123.9[a]		
C-3′	107.9	6.60 s (H_B)	
C-4′	145.1		6.60 (H_B)
C-5′	136.4		6.60 (H_B)
C-6′	144.2[c]		
C-7′	168.6		6.60 (H_B); 5.38 (glc H-3)
4,6-Gallagyl			
C-1	122.1[a]		
C-2	114.4		6.49 (H_C)
C-3	145.1[c]		
C-4	136.1		6.49 (H_C)
C-5	145.6		6.49 (H_C)
C-6	107.5	6.49 s (H_C)	
C-7	169.1		6.49 (H_C); 4.90 (glc H-4)
C-1′	124.2[a]		
C-2′	116.7		6.98 (H_D)
C-3′	143.7[c]		

TABLE 1. Continued.

	δ_C	δ_H (J Hz)	$^2J_{C\text{-}H}$ and $^3J_{C\text{-}H}$
C-4′	137.7		6.98 (H_D)
C-5′	145.7		6.98 (H_D)
C-6′	111.5	6.98 s (H_D)	
C-7′	168.2		6.98 (H_D); 4.40 (glc H-6)
C-1″	114.2[b]		
C-2″	148.3[d]		
C-3″	139.1[e]		
C-4″	136.8[f]		
C-5″	114.9[b]		
C-6″	126.0[a]		
C-7″	157.9[g]		
C-8″	114.9[b]		
C-9″	136.3[f]		
C-10″	138.8[e]		
C-11″	147.4[d]		
C-12″	114.2[b]		
C-13″	126.0[a]		
C-14″	158.4[g]		

[a–g]Assignments may be interchanged.

A characteristic yellow color of the aqueous solution and uv absorptions (λ max, MeOH) at 217, 260, and 379 nm suggested the presence of a gallagyl group in tannin **4**. The two higher-field signals in the ^{13}C-nmr spectrum at δ 157.9 and 158.4 without any cross-peak were assigned to the lactonic carbons of the gallagyl group (5). The two carbonyl carbons at δ 168.2 and 169.1 were each correlated with one glucose proton and one aromatic proton, at δ 4.40 (H-6 of glucose) and 6.98 (H-D) for the first of these and at δ 4.90 (H-4 of glucose) and 6.49 (H-C) for the second. Thus, the two ester carbonyls of the gallagyl group could be linked, as in the punicalin structure, to *O*-4 and *O*-6 of the glucose, leading to a strong inequivalence for the two glucose protons at position 6 (δ 4.40 and 2.10).

Finally, the ester carbon signals at δ 168.1 (C-7) and 168.6 (C-7′) each showed correlations to two protons, one aromatic and one glucose-linked, at δ 6.40 (H-A) and δ 5.15 (C-7) and at δ 6.60 (H-B) and δ 5.38 (C-7′). These two carbonyl groups, assigned to a hexahydroxydiphenoyl (HHDP) moiety, were linked to *O*-2 and *O*-3 of the glucose unit of **4**.

Other cross-peaks were evident in the long-range ^{1}H-^{13}C-nmr spectrum of tannin **4**, and allowed the assignment of aromatic carbons. Correlations were observed between H-2 and C-3 and C-4 for the galloyl group, between H-A and C-1, C-4, and C-5 as well as between H-B and C-1′, C-4′, and C-5′ for the 2,3-HHDP group, and finally for the gallagyl group between H-C and C-2, C-4, and C-5, and between H-D and C-2′, C-4′, and C-5′.

The structure of combreglutinin [**4**] was confirmed by partial acid hydrolysis (5), which produced the yellow punicalin [**2**] by loss of 1 α-galloyl and 2,3-hexahydroxydiphenoyl groups, and punicalagin [**3**] by loss of only 1 α-galloyl group, together with ellagic acid and gallic acid. Inasmuch as punicalin [**2**] and punicalagin [**3**] have been shown to possess two and three atropisomeric centers, respectively, with (*S,S*)-configuration for the gallagyl group and (*S*) for the hexahydroxydiphenoyl group (5), the structure of combreglutinin was determined as 1 α-*O*-galloyl-2,3-(*S*)-hexahydroxydiphenoyl-4,6-(*S,S*)-gallagyl-D-glucose, **4**. A similar structure to combreglutinin [1-(α)-*O*-galloylpunicalagin] was mentioned without supporting chemi-

cal and physical data in *Terminalia calamansanai* (8). Inhibition of hepatitis B virus surface antigen by tannins **1–4** is under investigation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were measured with a Perkin-Elmer 141 polarimeter and fabms with a VG Analytical ZAB-HF mass spectrometer. ^{1}H- (300.13 MHz) and ^{13}C- (75.45 MHz) nmr spectra were recorded on a Bruker AC-300 spectrometer, with TMS as internal standard. Chemical shifts are reported on the δ ppm scale. Tlc was performed on precoated Kieselgel 60 F_{254} plates (0.20 mm, Merck) with CH_2Cl_2-MeOH-HCl (85:15:0.5), and the zones detected with $FeCl_3$ reagent.

PLANT MATERIAL.—*C. glutinosum* was collected at M'Bour, near Dakar in Senegal, in December 1992. A voucher specimen was deposited at the Herbarium of the Muséum National d'Histoire Naturelle, Paris, France.

EXTRACTION AND ISOLATION.—The air-dried ground leaves (400 g) of *C. glutinosum* were extracted successively with CH_2Cl_2 and MeOH in a Soxhlet. After removal of solvent under reduced pressure, the MeOH extract (40 g) was fractionated by chromatography on Sephadex LH-20. Elution with H_2O containing increasing amounts of MeOH afforded successively 2,3-(*S*)-hexahydroxydiphenoyl-D-glucose [**1**] (0.4 g), punicalin [**2**] (0.12 g), punicalagin [**3**] (8 g), and combreglutinin [**4**] (1.6 g).

2,3-(*S*)-HEXAHYDROXYDIPHENOYL-D-GLUCOSE [**1**].—Amorphous powder; uv (MeOH) λ max (log ε) 207 (4.49), 260 (4.24), 364 (sh, 3.43) nm.

Isomer **1α**, ^{1}H nmr (Me_2CO-d_6+D_2O) δ 5.35 (1H, d, *J*=3.5 Hz, H-1), 4.85 (1H, dd, *J*=3.5 and 9 Hz, H-2), 5.22 (1H, t, *J*=9 Hz, H-3), 3.73 (1H, t, *J*=9 Hz, H-4), 3.90 (1H, m, H-5), 3.87 (1H, m, H-6) and 3.37 (1H, m, H-6), 6.58 (1H, s, H_A), 6.64 (1H, s, H_B).

Isomer **1β**, ^{1}H nmr (Me_2CO-d_6+D_2O) δ 4.91 (1H, d, *J*=9 Hz, H-1), 4.63 (1H, t, *J*=9 Hz, H-2), 4.95 (1H, t, *J*=9 Hz, H-3), 3.69 (1H, t, *J*=9 Hz, H-4), 3.50 (1H, m, H-5), 3.87 (1H, m, H-6) and 3.37 (1H, m, H-6), 6.59 (1H, s, H_A), 6.98 (1H, s, H_B), lit. (2).

PUNICALIN [**2**].—Yellow amorphous powder, $[\alpha]_D^{20}$ −80° (*c*=0.5, MeOH) [lit. −80°, *c*=1, H_2O (4) and −81.1°, *c*=0.6, H_2O (5)]. Uv (MeOH) λ max (log ε) 216 (4.87), 258 (4.83), 379 (4.15) nm.

Isomer **2α**, ^{1}H nmr (CD_3OD) δ 4.77 (1H, d, *J*=3 Hz, H-1), 3.18 (1H, dd, *J*=3 and 9 Hz, H-2), 3.57 (1H, t, *J*=9 Hz, H-3), 4.18 (1H, t, *J*=9 Hz, H-4), 2.85 (1H, br t, *J*=9 Hz, H-5), 2.23 (1H, br d, *J*=11 Hz, H-6a) and 3.92 (1H, dd, *J*=9 and 11 Hz, H-6b), 6.60 (1H, s, H_C), 6.82 (1H, s, H_D). ^{13}C nmr (CD_3OD) δ (selected) 91.5 (C-1), 72.6 (C-2), 72.3 (C-3), 74.3 (C-4), 69.0 (C-5), 64.8 (C-6); 4,6-gallagyl 171.2 (C-7), 169.7 (C-7′); δ-lactones 160.2, 160.1.

Isomer **2β**, ^{1}H nmr (CD_3OD) δ 4.15 (1H, d, *J*=9 Hz, H-1), 3.01 (1H, t, *J*=9 Hz, H-2), 3.25 (1H, t, *J*=9 Hz, H-3), 4.30 (1H, t, *J*=9 Hz, H-4), 2.34 (1H, br t, *J*=9 Hz, H-5), 2.12 (1H, br d, *J*=11, H-6a) and 3.86 (1H, dd, *J*=9 and 11 Hz, H-6b), 6.80 (1H, s, H_C), 6.98 (1H, s, H_D). ^{13}C nmr (CD_3OD) δ (selected) 97.5 (C-1), 75.6 (C-2), 76.4 (C-3), 74.3 (C-4), 73.0 (C-5), 65.2 (C-6); 4,6-gallagyl 171.2 (C-7), 169.5 (C-7′); δ-lactones 160.1, 159.9

PUNICALAGIN [**3**].—Yellow amorphous powder, $[\alpha]_D^{20}$ −110° (*c*=0.5, MeOH) [lit. −181°, *c*=1, H_2O (4) and −162.2°, *c*=1.3, H_2O (5)]. Uv (MeOH) λ max (log ε) 217 (4.89), 261 (4.85), 375 (4.13) nm.

Isomer **3α**, ^{1}H nmr (Me_2CO-d_6+D_2O) δ 5.12 (1H, d, *J*=3 Hz, H-1), 4.82 (1H, dd, *J*=3 and 9 Hz, H-2), 5.20 (1H, t, *J*=9 Hz, H-3), 4.77 (1H, t, *J*=9 Hz, H-4), 3.22 (1H, br t, *J*=9 Hz, H-5), 2.10 (1H, br d, *J*=10 Hz, H-6a) and 4.06 (1H, br dd, *J*=9 and 10 Hz, H-6b), 6.51 (1H, s, H_A), 6.58 (1H, s, H_B), 6.66 (1H, s, H_C), 6.98 (1H, s, H_D). ^{13}C nmr (Me_2CO-d_6+D_2O) δ (selected) 90.1 (C-1), 74.3 (C-2), 76.4 (C-3), 71.0 (C-4), 66.7 (C-5), 64.1 (C-6); 2,3-(*S*)-hexahydroxydiphenoyl 168.8 (C-7), 168.4 (C-7′); 4,6-gallagyl 169.4 (C-7), 168.0 (C-7′); δ-lactones 157.8, 158.3.

Isomer **3β**, ^{1}H nmr (Me_2CO-d_6+D_2O) δ 4.69 (1H, d, *J*=9 Hz, H-1), 4.63 (1H, t, *J*=9 Hz, H-2), 4.89 (1H, t, *J*=9 Hz, H-3), 4.80 (1H, t, *J*=9 Hz, H-4), 2.66 (1H, br t, *J*=9 Hz, H-5), 2.19 (1H, br d, *J*=10 Hz, H-6a) and 4.13 (1H, br dd, *J*=9 and 10 Hz, H-6b), 6.52 (1H, s, H_A), 6.58 (1H, s, H_B), 6.72 (1H, s, H_C), 7.00 (1H, s, H_D). ^{13}C nmr (Me_2CO-d_6+D_2O) δ (selected) 94.3 (C-1), 76.7 (C-2), 78.8 (C-3), 70.7 (C-4), 72.5 (C-5), 64.1 (C-6); 2,3-(*S*)-hexahydroxydiphenoyl 168.6 (C-7), 168.2 (C-7′); 4,6-gallagyl δ 169.1 (C-7), 167.9 (C-7′); δ-lactones 157.8, 158.3.

COMBREGLUTININ [**4**].—Brown amorphous powder, $C_{55}H_{32}O_{34}$, $[\alpha]_D^{20}$ −60° (*c*=0.33, MeOH). Negative fabms *m/z* 1235 $[M-H]^-$, 427, 320, 213. Hr (−) fabms *m/z* $[M-H]^-$ 1235.0653, calcd for $C_{55}H_{31}O_{34}$, 1235.0697. Uv (MeOH) λ max (log ε) 217 (4.89), 260 (4.82), 379 (4.01) nm. For nmr data, see Table 1.

PARTIAL ACID HYDROLYSIS OF COMBREGLUTININ [**4**].—A solution of **4** (200 mg) in 1 N H_2SO_4 was

heated at 90° for 4 h. After cooling, the resulting pale yellow needles (60 mg) were collected by filtration and identified as ellagic acid by comparison of its ir spectrum with that of an authentic sample. The filtrate was then extracted with EtOAc. Tlc examination of this extract showed the presence of gallic acid. The aqueous layer was adjusted to pH 5 with $NaHCO_3$, and further subjected to Sephadex LH-20 chromatography using H_2O with increasing amounts of MeOH to give punicalin [**2**] (75 mg) and punicalagin [**3**] (5 mg).

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