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ISOFLAVONE GLYCOSIDES FROM *NEORAUTANENIA AMBOENSIS*[1]

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ABSTRACT.—Four novel isoflavone glycosides, ambonin (**2**), ambocin (**4**), neobanin (**6**), and neobacin (**8**), were isolated from *Neorautanenia amboensis*, and their structures were determined mainly by physical methods, especially high resolution nmr spectroscopy. The sugar sequence and interglycosidic linkage in ambonin (**2**) as well as the positions of the sugar substituents in neobanin (**6**) were determined utilizing the selective population inversion technique.

Neorautanenia species (Leguminosae) are used (1) as fish poisons in Central and Southern Africa, and systematic examination of the metabolites present in *Neorautanenia amboensis* Schinz has revealed a wealth of pterocarpans (2), a series of rotenoids (3), and a few isoflavanones. These isoflavonoids were the main constituents of the successive hexane and C_6H_6 extracts.

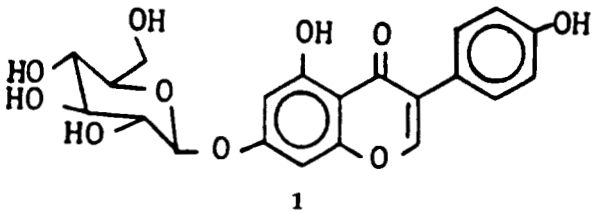
The final MeOH extract of the bark contained, in addition to sugars, amino acids, two 2-benzyldihydrobenzofuran glucosides (4), and the isoflavone glycosides **1-9**. Compound **1** was identified as genistin (5) by spectroscopic means. This article describes four new isoflavone glycosides, ambonin (**2**), ambocin (**4**), neobanin (**6**), and neobacin (**8**), which were isolated using counter current distribution and column chromatography. The structures were determined mainly by physical methods. The selective population inversion (spi) technique (6) was employed for determining the sugar sequence and the interglycosidic linkage in **2** and **4**, while the same technique established the substitution positions of the glucose and apiose moieties in **6** and **8**.

RESULTS AND DISCUSSION

The MeOH extract of the bark from the bulb yielded, after counter current distribution between EtOAc and H_2O, a fraction only soluble in the lower phase. Subsequent column chromatography on silica gel delivered **1** and separated the rest into two fractions: one consisting of **2** and **4**; the second containing **6** and **8**. The pure heptaacetates **3**, **5**, **7**, and **9** were obtained upon acetylation and column chromatography. Ambonin (**2**) and neobanin (**6**) were characterized by a light yellow color with tetra-azotized benzidine (7) and with diazotized 4-nitroaniline (8) and orange with Millon's reagent (9), while they were not chromogenic to diazotized sulfanilic acid (10). Ambocin (**4**) and neobacin (**8**) developed a red-brown color with tetra-azotized benzidine, grey with Millon's reagent, and orange with diazotized sulfanilic acid but were inactive towards 4-nitroaniline. All compounds and their acetates reacted poorly with perchloric acid-$FeCl_3$, and all gave a positive Molisch test. Enzymic hydrolysis with β-glucosidase in all instances gave the aglycones and two monosaccharides thus prohibiting determination of the sugar substitution pattern by selective removal of glucose.

Enzymic and acid hydrolysis of ambonin (**2**) afforded the aglycone daidzein (5) and two sugars. Glc of the derivatized hydrolysate allowed identification of one as glucose while the other had a retention time not corresponding to any of the standards on hand. A molecular mass of 842 was obtained for ambonin heptaacetate (**3**) by fdms with substantial fragmentation to ions at *m/z* 800 (loss of ketene, $H_2C{=}C{=}O$, 42), 782 (loss of

[1]A part of this work was presented at the Sixth Congress of the Academy of Pharmaceutical Sciences, Pretoria, 1985.



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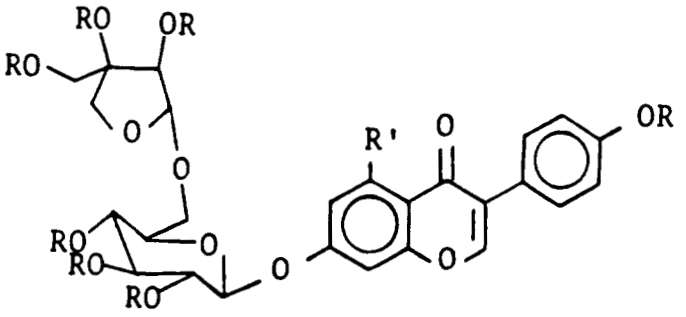
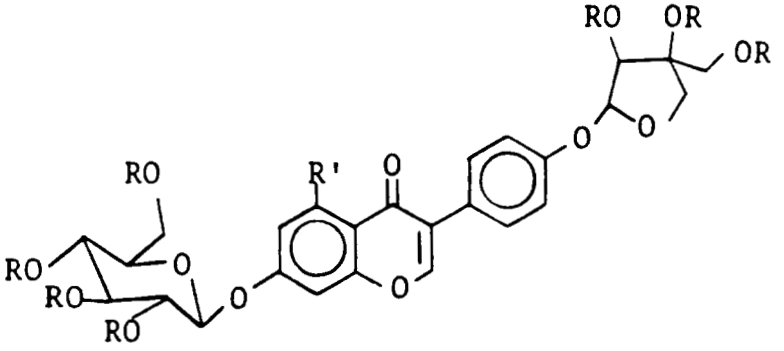


2 R=R′=H
3 R=Ac, R′=H
4 R=H, R′=OH
5 R=Ac, R′=OH



6 R=R′=H
7 R=Ac, R′=H
8 R=H, R′=OH
9 R=Ac, R′=OH

HOAc), 590 (-6×42), and 548 (-7×42). The dissaccharide unit was lost during eims which gave prominent peaks at *m/z* 296 (daidzein acetate) and 254 (daidzein) as well as peaks arising from the known RDA-fragmentation of the isolavone molecule.

The 500 MHz ^{1}H-nmr spectrum of ambonin heptaacetate (**3**) displayed the signals of one aromatic (δ 2.28) and six aliphatic acetate groups, while the resonances characteristic of the isoflavone moiety were clearly recognizable (Table 1). The signal of H-3′,5′ at low field (δ>7.1) suggested the presence of the aromatic acetyl function in position 4′, implying that the sugar unit was in position 7.

The heterocyclic region, δ 3.5-5.8, which integrated for 13 protons, was complex and not fully resolved in $CDCl_3$. The relative difference in chemical shift in acetone-d_6, however, enabled decoupling experiments, data from which along with proton-proton correlations completed the assignment of the proton resonances. The glucose moiety was defined by the resonances of a seven spin system. Irradiation of the signal of H-1″ (δ 5.67) resolved the doublet of doublets due to H-2″ into a doublet ($J_{2'',3''}$ 8.85 Hz), while

TABLE 1. ^{1}H-nmr Data of Compounds **3** and **7**[a]

Proton	Compounds		
	3 ($CDCl_3$)	**3** (acetone-d_6)	**7** ($CDCl_3$)
H-2	8,05	8,34	8,00
H-5	8,19	8,17	8,18
H-6	7,00	7,16	7,00
H-8	7,09	7,30	7,07
H-2′,6′	7,57	7,68	7,34
H-3′,5′	7,12	7,20	6,80
H-1″	5,19	5,67	5,18
H-2″	5,16	5,29	5,25
H-3″	5,31	5,44	5,33
H-4″	5,01	5,09	5,02
H-5″	3,90	4,31	3,90
H-6″a	3,57	3,68	3,58
H-6″b	3,75	3,89	3,75
H-1‴	4,96	5,06	4,97
H-2‴	5,40	5,42	5,39
H-4‴a	4,11	4,08	4,11
H-4‴b	4,23	4,28	4,23
H-5‴a	4,64	4,71	4,63
H-5‴b	4,77	4,76	4,77
4′-OAc	2,28	2,29	—
OAc	2,70; 2,04 2,04; 2,00 1,99; 1,98	2,06; 2,04 2,04; 2,00 1,99; 1,98	2,07; 2,04 2,04; 2,04 2,01; 1,99 1,98

[a]ppm from TMS at 500 MHz.

decoupling of H-2″ similarly simplified the triplet attributable to H-3″ to a doublet ($J_{3'',4''}$ 8.85 Hz) and the doublet representing H-1″ to a singlet. Decoupling of H-3″ caused H-4″ and H-2″ to appear as doublets ($J_{4'',5''}$10.0 Hz and $J_{1'',2''}$7.5 Hz, respectively) while the signals of H-6″a, H-6″b, and H-4″ each changed upon irradiation of H-5″ from a doublet of doublets each ($J_{6''a,6''b}$11.2 Hz) to a doublet each. The cited coupling constants illustrated the overall axial orientation of these protons and, thus, the identity of this residue as D-glucose. The remaining six protons in this region did not form a coherent spin system. H-5‴a and H-5‴b appeared as a doublet each ($J_{5'''a,5'''b}$12.35 Hz) as did H-4‴a and H-4‴b ($J_{4'''a,4'''b}$10.0 Hz). H-1‴ and H-2‴ resonated as singlets. The absence of coupling between these vicinal protons indicated their *trans* position on the β-furanose ring (11).

The above-mentioned assignments and couplings were confirmed by a two dimensional homonuclear correlation, which also revealed a small ($J<1$ Hz) coupling between H-1‴ and H-2‴ and between H-4‴a and H-5‴a.

The ^{13}C-nmr spectrum of ambonin heptaacetate (**3**) contained, in addition to signals arising from seven acetate groups, the expected resonances of the isoflavone aglycone (12). The location of the aromatic acetyl group in position 4′ (as opposed to position 7) was supported by the resonance of C-3′,5′ at low field (δ 121.58). The signals of the carbon atoms could, with the exception of C-2″ and C-3″ where the proton signals overlap, all be assigned using two dimensional ^{13}C-^{1}H-heteronuclear correlations (Figure 1). These data (Table 2) were supported by the multiplicities of the carbon signals in the coupled ^{13}C-nmr spectrum. The resonances of the anomeric carbon atoms of the glucose and apiose moieties in **3** appeared at δ 98.36 and 106.04, respectively. A

TABLE 2. ^{13}C-nmr Data of Compounds **3**, **5**, **7**, and **9**[a]

Carbon	Compounds			
	3	**5**	**7**	**9**
C-2	153,38	151,11	152,94	151,73
C-3	129,53	125,63	125,03	122,51[b]
C-4	175,39	177,62	176,17	178,80
C-4a	124,38	105,71	123,29	106,31
C-5	128,04	159,23[b]	127,98	160,10[c]
C-6	115,83	95,81	115,69	97,03
C-7	160,61	159,82[b]	160,56	160,80[c]
C-8	104,10	92,74	104,00	94,22
C-8a	157,43	154,52	157,43	155,81
C-1′	120,10	120,61	119,92	121,30[b]
C-2′,6′	130,08	127,52	130,22	128,83
C-3′,5′	121,58	119,44	115,61	114,41
C-4′	150,59	148,00	156,43	155,21
glucose				
C-1″	98,36	98,61	98,27	99,54
C-2″	72,70[b]	70,91[c]	72,66[b]	71,83[d]
C-3″	71,14[b]	70,63[c]	71,09[b]	71,51[d]
C-4″	68,75	67,19	68,69	67,83
C-5″	73,83	71,81[c]	73,70	72,80[d]
C-6″	66,44	64,72	66,40	65,91
apiose				
C-1‴	106,04	103,90	105,98	105,13
C-2‴	76,15	74,61	76,99	75,63
C-3‴	83,76	82,01	83,76	83,00
C-4‴	72,69	69,29	72,55	70,30
C-5‴	63,10	61,30	63,06	62,22

[a]ppm from TMS in $CDCl_3$ at 125,75 MHz.
[b,c,d]Resonances bearing the same superscript in any column are interchangeable.

β-anomeric configuration was suggested (13) for apiose by the resonance of C-1‴ at low field, but the signal of C-1″ was not indicative of the anomeric configuration of the glucose moiety.

The sugar sequence and the interglycosidic linkage were determined using the spi technique (6). This technique, utilizing long distance coupling, reveals atoms three bonds apart. Application of a selective pulse to the singlet signal of H-1‴ affected the carbon resonances at δ 83.76 (C-3‴), 72.69 (C-4‴), and 66.44 (C-6″) proving that the mentioned carbon atoms were each three bonds away from H-1‴, thus establishing apiose as the terminal sugar linked at C-1 to C-6 of glucose. The β-anomeric configuration for glucose was proposed on the basis of the proton coupling constant as well as the observation that ambonin (**2**) was hydrolyzed by β-glycosidase. The above evidence established the structure of **2**.

The ir, ms, ^{1}H-nmr, and ^{13}C-nmr spectral patterns of ambocin (**4**) and its heptaacetate (**5**) were very similar to those of **2** and **3**, respectively. The only differences were an increased molecular mass (+16 amu), an aromatic AX system instead of the ABX system in the ^{1}H-nmr spectrum, and differences in the chemical shifts of C-4a, C-5, C-6, and C-8 (Table 2). These data implied the presence of an hydroxyl group in position 5, which was confirmed by acid hydrolysis of **4** and characterization of the aglycone as genistein.

Hydrolysis of neobanin (**6**) yielded the same compounds as ambonin (**2**). The ^{1}H- and ^{13}C-nmr spectra of neobanin heptaacetate (**7**) were almost identical to those of am-

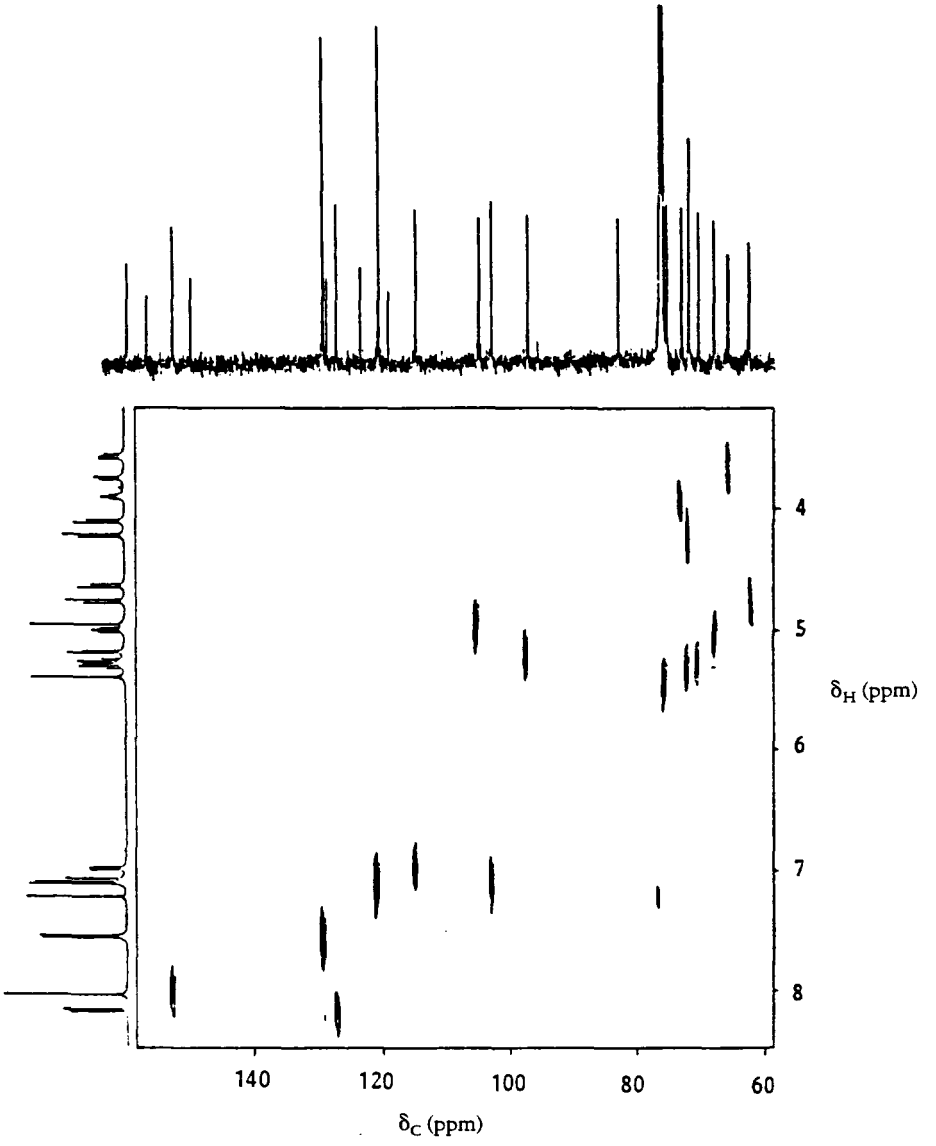


FIGURE 1. Two dimensional ^{13}C-^{1}H correlation (HETCOR) of ambonin heptaacetate (**3**).

bonin heptaacetate (**3**) (Tables 1 and 2); the only differences in the ^{1}H-nmr spectrum were the presence of seven aliphatic acetoxy signals instead of one aromatic and six aliphatic signals and the diamagnetic shift of the resonance of H-3′,5′ of 0.32 ppm; the only differences in the ^{13}C-nmr spectrum were the diamagnetic shift ($\Delta\delta$ −6 ppm) of the resonance due to C-3′,5′ and the paramagnetic shift ($\Delta\delta$ 6 ppm) of the signal from C-4′. This evidence supported an isoflavone structure substituted with a sugar unit in each of positions 7 and 4′. The spi technique revealed the positions of the carbohydrate residues. Irradiation of the signal of H-1‴ by this method affected the carbon resonances at δ 72.55 (C-4‴), 83.76 (C-3‴), and 156.43 (C-4′) proving that apiose was the sugar in position 4′.

The ir, ms, ^{1}H-nmr, and ^{13}C-nmr spectra of neobacin (**8**) and its heptaacetate (**9**) corresponded closely to those of **6** and **7**, respectively. The only differences were the increased molecular mass (+16 amu), the aromatic AX system in the place of an ABX sys-

tem in the ^{1}H-nmr spectrum, and changes in the chemical shifts of C-4a, C-5, C-6, and C-8 (Table 2). These data indicated the presence of a hydroxyl group in position 5 which was confirmed by acid hydrolysis of **8** and identification of the aglycone as genistein.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Leitz microscope and are uncorrected. Ir spectra were recorded for KBr-discs on a Nicolet 5 DX ft-ir, while optical rotations were measured on a Officine Galileo polarimeter. Mass spectra were recorded on a VG 70-70E mass spectrometer, while fdms was done by Dr. U. Rapp, Varian MAT GmbH, Bremen. ^{1}H-nmr spectra were recorded on a Bruker WP 80 instrument at 80 MHz, and high resolution ^{1}H-nmr homo and heteronuclear correlations and spi detections were done on a Bruker WM 500 instrument at 500 MHz for ^{1}H and 125.75 MHz for ^{13}C. TMS was used as internal standard and $CDCl_3$ as solvent, unless otherwise specified.

ISOLATION OF THE COMPOUNDS.—Plant material was authenticated by botanist P. van Wyk (4). Preparation and extraction of the plant material, counter current separation of the extract, and chromatographic conditions were as previously described (4). A part (80 g) of fraction 1 (4) of the lower phase was chromatographed over silica gel (Me_2CO-MeOH, 4:1) to deliver sucrose (8 g), glucose (ca. 1 g), amino acids, and a fraction containing the isoflavonoid glycosides. Column chromatography of the last mentioned fraction over silica gel ($CHCl_3$-Me_2CO-MeOH-H_2O, 20:20:9:1) gave genistin (**1**) (11 mg) and fractions A and B. Fraction A yielded upon column chromatography (C_6H_6-Me_2CO-MeOH-H_2O, 20:20:9:1) ambocin (**4**) (175 mg) and ambonin (**2**) (3.25 g), while fraction B delivered neobacin (**8**) (110 mg) and neobanin (**6**) (2,75 g).

Ambonin (**2**).—Compound **2** was obtained as fine white rosettes (Me_2CO-H_2O, 2:3), mp 225-227°; $[\alpha]_D$ −71.2° (*c* 0.014, H_2O); Rf 0.50 (C_6H_6-Me_2CO-MeOH-H_2O, 20:20:9:1) and 0.54, 0.73 (Whatman No 1).

Ambonin heptaacetate (**3**).—Compound **3** provided fine white rosettes (C_6H_6), mp 154-155°; Rf 0.54 ($CHCl_3$-Me_2CO, 9:1) [found: C. 56.94; H, 4.99, $C_{40}H_{42}O_{20}$ requires C, 57.00; H, 5.02]; fdms *m/z* 843 $(M+H)^+$, 842 (M^+), 620, 590, 548, 502, 456, 445, 424, 398, 254; ir ν max cm^{-1} 1000, 1200, 1410, 1500, 1550, 1680, 1760, 2420, 3300.

Ambocin (**4**).—Compound **4** was a colorless glass, $[\alpha]_D$ −36.5° (*c* 0.011, H_2O), Rf 0.56, 0.65 (Whatman No 1).

Ambocin heptaacetate (**5**).—Compound **5** developed a poor green color with perchloric acid-$FeCl_3$ spray reagent, Rf 0.61 ($CHCl_3$-Me_2CO, 9:1).

Neobanin (**6**).—Compound **6** formed fine rosettes ($CHCl_3$-MeOH, 1:1), mp 211-212°; $[\alpha]_D$−72.6° (*c* 0.018, H_2O); Rf 0.50 (C_6H_6-MeOH-Me_2CO-H_2O, 20:20:9:1) and 0.55, 0.72 (Whatman No 1).

Neobanin heptaacetate (**7**).—Compound **7** gave fine rosettes (C_6H_6), mp 160-161°, Rf 0.34 ($CHCl_3$-Me_2CO, 9:1) (found: C, 57.03; H, 5.10, $C_{40}H_{42}O_{20}$ requires C, 57.00; H, 5.02); ir ν max cm^{-1} 1010, 1200, 1400, 1500, 1660, 1680, 1760, 2420, 3300.

Neobacin (**8**).—Compound **8** was a colorless glass, $[\alpha]_D$ −38.2° (*c* 0.011, H_2O) Rf 0.51, 0.64 (Whatman No 1).

Neobacin heptaacetate (**9**).—Compound **9** developed a poor green color with perchloric acid-$FeCl_3$ spray reagent, Rf 0.22 ($CHCl_3$-Me_2CO, 9:1).

HYDROLYSIS OF THE GLYCOSIDES.—(a) β-Glucosidase (SIGMA, 50 mg) was added to a solution of **2**, **4**, **6**, or **8** (150 mg) in NaOAc-HOAc buffer solution (pH 5.0, 3 ml) and allowed to stand at 37° for 48 h after which it was extracted with Et_2O. The extract was washed (saturated $NaHCO_3$ and H_2O), dried ($MgSO_4$), and evaporated in vacuo. The product was purified by column chromatography and in each instance identified as the aglycone by spectral methods. (b) A solution of the glycoside (100 mg) in 1N HCl (0.5 ml) was heated at 60° for 1 h after which it was extracted with Et_2O and worked up as above. The products were identified by spectral means.

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