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NEW FLAVONOIDS FROM SUGARCANE (*SACCHARUM*)

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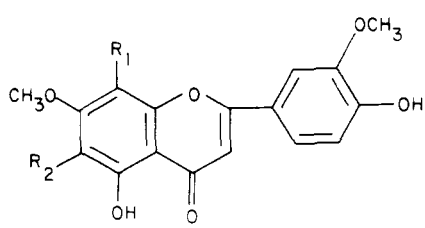
ABSTRACT.—Two new flavonoid glycosides, tricin 7-(2″-rhamnosyl)-α- galacturonide (**2**) and orientin 7,3′-dimethyl ether (**4**), were isolated from sugarcane (*Saccharum*) mill syrup. In addition, five other flavonoid C- and O-glycosides were identified, including swertisin (**3**), isoorientin 7,3′-dimethyl ether (**5**), schaftoside (**6**), isoschaftoside (**7**), and tricin 7-glucoside (**1**). Their structures were established using spectroscopic and chemical evidence.

The leaf flavonoid patterns of several species of *Saccharum* have been studied for taxonomic and evolutionary purposes in recent years (1-4). There is also an economic interest in identifying the flavonoids present in commercial raw sugar due to their contribution to the color in the unrefined product. However, only a few flavonoids have thus far been isolated and identified in cane-sugar process streams (5-7).

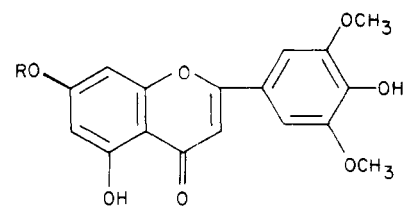
The commercial cane employed in the present study represents hybrid varieties cultivated in North Queensland, Australia. About 80% of the germplasm of these hybrids originated from *Saccharum officinarum* L., in particular the clones Badila and Korpi, while the remaining 20% is derived from wild taxa, notably *Saccharum spontaneum* L., to impart vigor and disease resistance (8). The crude pigment from combined mill syrups yielded seven flavonoid C- and O-glycosides, including tricin 7-glucoside (**1**), the previously unreported tricin 7-(2″-rhamnosyl)-α- galacturonide (**2**), and orientin 7,3′-dimethyl ether (**4**). Spectroscopic and chemical evidence is presented here for the structures of **1**, **2**, and **4**.

RESULTS AND DISCUSSION

Hydrolysis of **2** yielded tricin, rhamnose, and galacturonic acid. Substitution at the 7-OH was indicated by the position of Band I at 425 nm in the "NaOAc" uv spectrum relative to that in the "NaOMe" spectrum (412 nm), and by the absence of Band III in the "NaOMe" spectrum (9). Permethylation (PM) produced two products having molecular ions at *m/e* 764 and 750 (*m/e* 765 and 751 in ci-ms). The difference of 14 mass units is expected for the methyl ester and free acid forms, respectively, of the galac-

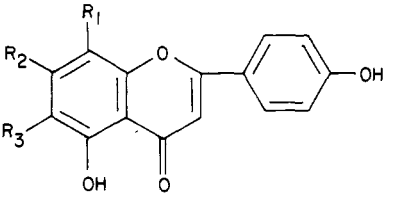


1 R=glc
2 R=(2″-rha)-α-galur



4 R_1=glc, R_2=H
5 R_1=H, R_2=glc

turonic acid moiety. A base peak at *m/e* 189 for both PM products is consistent with a permethylated terminal rhamnosyl moiety. The ^{1}H-nmr spectrum of the TMSi ether of **2** exhibited an H-1″ doublet at δ 5.45 with a *J* value of about 3 Hz, indicating an α- rather than a β-linked galacturonic acid. Synthetic α- and β-glucuronides have been reported to have coupling constants of *J*=3.5-3.6 Hz and 6.8-8.2 Hz, respectively (10). Other ^{1}H-nmr signals were consistent with the proposed structure (see Experimental section).



3 R_1=CH_3, R_2=H, R_3=glc
5 R_1=H, R_2=ara, R_3=glc
7 R_1=H, R_2=glc, R_3=ara

Information about the position of the interglycosidic linkage in **2** was provided by ^{13}C-nmr spectroscopy. Assignments for carbon atoms on the aglycone nucleus and terminal rhamnose were made by comparison with analogs (11), leaving six unassigned signals, presumably due to the galacturonic acid carbons. Because no reference ^{13}C-nmr data were available for either flavonoid galacturonides or glycosylated hexuronides, recourse was made to O-glucosyl, O-galactosyl, and O-glucuronide models (11,12), as well as to known shifts in glycosyl signals due to terminal rhamnosylation (11). Using these models to predict shifts for a 7-O-rhamnosylgalacturonide, it was found that rhamnosylation at the 2″ position was clearly favored over substitution at other sites on the galacturonic acid moiety. Moreover, there was good agreement between the spectrum of **2** and that calculated for a 7-O-(2″-O-α-L-rhamnopyranosyl)-O-α-D-galactopyranosyluronide from the data published for kaempferol 4′-O-(2″-O-α-L-rhamnopyranosyl)- (6″-O-α-L-rhamnopyranosyl)-O-β-D-galactopyranoside and kaempferol 3-O-robinoside 7-rhamnoside (13). This calculation involved such steps as adjusting for the effect of a 6″-carboxyl (12) and attachment to a 7-hydroxyl position rather than to a 4′-hydroxyl (11). Accordingly, the complete description of **2** is tricin 7-O-(2″-O-α-L-rhamnopyranosyl)-O-α-D-galactopyranosyluronide.

Orientin 7,3′-dimethyl ether (**4**) and tricin 7-O-glucoside (**1**) were not satisfactorily separated, each always remaining contaminated with the other; so rather than lose more material, characterization was undertaken on the mixture. In the uv spectra of **1** and **4**, the presence of Band I in NaOAc at greater wavelength than in NaOMe (9), and the absence of Band III in NaOMe, suggested substitution at the 7-position in both compounds. Perdeuteromethylation (PDM) yielded two products, one of which was identified as the PDM ether of **1**. The other had a molecular ion at *m/e* 578 and base peak at M-184, consistent with a PDM C-hexosyl di-O-methylluteolin structure. The high intensity M^+ and the absence of M-18 and M-34 peaks indicated that C-glycosylation was at the 8-position (14). Hydrolysis of the mixture of **4** and **1** in weak acid yielded tricin, glucose, and "starting material," while hydrolysis of available isoorientin 7,3′-dimethyl ether (**5**) in strong acid provided, in addition to **5**, a product with mass spectral and chromatographic properties identical to those of **4**. Since **5** and **4** should be interconvertible in strong acid via the Wessely-Moser rearrangement, the identity of **4** as orientin 7,3′-dimethyl ether is confirmed.

Compounds **2** and **4** are newly reported for *Saccharum*. The presence of C-glycosides and 7-O-glycosides and hexuronides in combined mill syrups from *Saccharum* is not un-

expected because such compounds would be expected to survive the conditions of heat, mild acidity, and alkalinity encountered in cane sugar processing.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Spectra were recorded with the following instruments: Absorption spectra, Beckman model 34; ^{1}H-nmr, Varian EM 390 90 MHz; ^{13}C-nmr, Brüker WH-90 22.6 MHz; mass spectra, DuPont 490 and AEI MS 902. Ei-ms were run with direct inlet probe at 70 eV, and ci-ms were run with NH_3. (For permethylation and purification of PM and PDM derivatives, see references 11 and 14.) Sugars were chromatographed on cellulose in EtOAc-pyridine-HOAc-H_2O (36:36:7:21) and visualized with aniline phthalate.

PLANT MATERIAL.—The sugarcane utilized herein consists of a broad spectrum of commercial hybrids sampled by CSR Ltd. from North Queensland, Australia, in mid-growing season, 1981. About 80% of the germplasm originates from *S. officinarum*, mainly Badila and Korpi clones, with the remaining 20% being derived from wild taxa, primarily *S. spontaneum* (8).

EXTRACTION AND REMOVAL OF SUCROSE.—The combined mill syrup and crude pigment were obtained in the following manner. In factory operations, the sugarcane juice was clarified by bringing the solution to pH 8.2 with lime, heating to just above boiling (*ca.* 103°), then filtering the solution and concentrating the filtrate to give mill syrup. The mill syrups from several raw sugar factories in North Queensland were combined to give the material that was subjected to ion exchange column chromatography (Rohm and Haas XAD-2) by standard procedures (7) in order to separate sucrose and inorganic constituents from the pigment material. The crude pigment was recovered by final elution of the XAD-2 column, then freeze-dried to give a deep brown powder.

CHROMATOGRAPHY.—Pigment (20 g) was dissolved in 50% MeOH, mixed with 25 g Polyclar, then dried, ground, and loaded onto a Polyclar column (7.5×45 cm) packed in EtOAc-MeOH (9:1). Elution was initiated with EtOAc-MeOH (9:1), gradually increasing the proportion of MeOH to 100%, followed by MeOH-H_2O with increasing proportion of H_2O. Similar fractions were combined and purified over Sephadex LH-20 eluted with MeOH-H_2O, followed by preparative paper chromatography.

TRICIN 7-O-(2″-O-RHAMNOSYL)-GALACTURONIDE (**2**).—Rf *t*-BuOH-HOAc-H_2O (3:1:1), 0.33, 15% HOAc, 0.31. Color on paper under uv (366 nm), purple; uv/NH_3, yellow; uv/Naturstoffreagenz-A. Carl Roth, Germany (NA), yellow; uv λ max (MeOH) 347, 265, 245sh; NaOMe, 412, 256sh, 250; $AlCl_3$, 390, 365sh, 303sh, 262; $AlCl_3$/HCl, 387, 365, 303sh, 265; NaOAc, 425, 355, 295sh, 257; NaOAc/H_3BO_3, 349, 265. ^{1}H-nmr (as TMS ether in CCl_4), δ 7.1 (s, 2H, H-2′, 6′), 6.6 (d, 1H, H-8), 6.5 (s, 1H, H-3), 6.4 (d, 1H, H-6), 5.45 (d, $J \leq 3$, 1H, H-1″), 4.75 (s, 1H, H-1‴), 3.91 (s, 6H, 3′, 5′-OMe), 3.57-4.33 (m, sugar protons), 1.10 (d, 3H, rha-CH_3); ei-ms (PM ether, methyl ester) *m/z* (%) 764 (M^+, 51), 732, 358, 189 (100); ci-ms *m/z* 765 (M+H, 42), 733, 359, 189 (100); ei-ms (PM ether, free acid) *m/z* (%) 750 (M^+, 14), 732, 543, 358, 189 (100); ci-ms *m/z* (%) 751 (M+H, 16), 733, 359, 189 (100); ^{13}C-nmr (DMSO-d_6) 17.9 (C-6‴), 56.4 (3′, 5′-OCH_3), 68.3 (C-4″), 70.4, 71.9 (C-2″, C-3‴, C-4‴, C-5‴), 73.6 (C-3″), 76.5 (C-5″), 77.3 (C-2″), 94.5 (C-8), 97.5 (C-6), 99.4 (C-1″), 100.6 (C-1‴), 103.9 (C-10), 104.6 (C-3, 2′, 6′), 120.1 (C-1′), 140.2 (C-4′), 148.2 (C-3′, 5′), 156.9 (C-9), 161.0 (C-5), 162.6 (C-7), 164.1 (C-2), *ca.* 172 (C-6″), 181.9 (C-4). Calculated figures for disaccharide moiety (see Discussion section): 100.2 (C-1″), 77.9 (C-2″), 72.9 (C-3″), 68.3 (C-4″), 75.2 (C-5″), 171 (C-6″); 100.2 (C-1‴), 70.3 and 72.1 (C-2‴, 3‴, 4‴, 5‴), 17.8 (C-6‴).

HYDROLYSIS OF **2**.—Hydrolysis in 0.1 N TFA at 100° for 1 h produced no detectable change. 2 N HCl for 1 h yielded some tricin (uv, ms, and co-tlc with authentic sample in three solvents), considerable amount of a partial hydrolyzate (tentatively identified as tricin 7-O-galacturonide), and rhamnose and galacturonic acid (*ca.* 2:1, co-tlc of aqueous layer with standard sugars). The partial hydrolyzate compound: color under uv (366 nm), purple; uv/NH_3, yellow or yellow-brown; uv/NA, yellow; absorption spectra: λ max (MeOH) 347, 302sh, 265, 245; NaOMe, 420, 290sh, 259; $AlCl_3$, 390, 370sh, 295sh, 268, $AlCl_3$/HCl, 390, 370sh, 295sh, 270; NaOAc, 424, 348, 260, 245; NaOAc/H_3BO_3, 348, 262, 245.

ORIENTIN 7,3′-DIMETHYL ETHER (**4**) AND TRICIN 7-O-GLUCOSIDE (**1**).—Compounds **4** and **1** were characterized as a mixture, and **4**, obtained by hydrolysis of **5**, gave an Rf in *t*-BuOH-HOAc-H_2O (3:1:1) of 0.39. Color on paper under uv of the mixture of **1** and **4** (366 nm), purple; uv/NH_3, yellow-green (**4**) and yellow (**1**); uv/NA, yellow; absorption spectra: λ max (MeOH) 345, 267sh, 250sh; NaOMe, 398 (incr.); $AlCl_3$, 384, 358, 300sh, 268sh, 256; $AlCl_3$/HCl, 383, 355, 300sh, 270sh, 259; NaOAc, 419, 347, 296sh, 257sh; NaOAc/H_3BO_3, 343, 260sh; ei-ms of **1** (PDM ether) *m/z* (%) 594 (M+, 64), 364 (100), 230, 196, 161, ei-ms of **4** (PDM ether) *m/z* (%) 578 (M+, 58), 394 (100), 376.

HYDROLYSIS OF **4** AND **1**.—Hydrolysis with 0.1 N TFA for 1.5 h yielded tricin (color reactions, ms, co-tlc with authentic sample in two solvents), glucose (co-tlc of aqueous layer with sugar standards), and unreacted **4**. Hydrolysis of **5** in 2 N HCl for 2 h yielded, in addition to **5**, a spot with a Rf value lower in 15% HOAc. When isolated, this compound proved identical (ms, color reactions, co-tlc in two solvents) to **4**.

ISOORIENTIN 7,3'-DIMETHYL ETHER (**5**), SCHAFTOSIDE (**6**), AND ISOSCHAFTOSIDE (**7**).—Color reactions, uv, co-tlc with authentic samples, and co-tlc of PM ethers with PM ethers of authentic samples established the identities of these compounds. ^{1}H-nmr, including benzene-induced shifts, was also used to elucidate **5**, and **6** and **7** were shown to be interconvertible via the Wessely-Moser rearrangement in 2 N HCl.

SWERTISIN (**3**).—Compound **3** was identified by color reactions, uv, ^{1}H-nmr (including benzene-induced shifts), and ms of the PDM ether.

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