7. These diterpenes probably belong to the *ent*-beyerene series, as the optical rotations of **2–4** were the same as those of the known compounds and those of **6**, **8** and **9** had the same signs.

The isolation of the *ent*-beyerene derivatives showed that *N. anomala* may be somewhat isolated in the genus, while the presence of **10** indicated the relationship to the other species. However, compounds like **10** have also been isolated from a few other genera of the tribe. An *ent*-beyerene derivative has been reported previously in the tribe only from a *Baccharis* species [8].

EXPERIMENTAL

The air-dried plant material collected in Transvaal (voucher 81/280, deposited in the Herbarium of the Botanic Research Institute, Pretoria), was extracted with Et_2O–petrol (1:2) and the resulting extracts were separated by CC (Si gel) and repeated TLC (Si gel). Compounds were identified by comparing the ^1H NMR spectra with those of authentic material. The roots (5 g) afforded 2 mg **10**, while the aerial parts (30 g) gave 4 mg coumarin, 20 mg **1**, 10 mg **3**, 6 mg **4**, 3 mg **5** (Et_2O–petrol, 3:1), 11.5 mg **7** (Et_2O–petrol, 3:1), 15 mg **9** (Et_2O–petrol, 1:10) and 1 mg **11**.

3α-Hydroxystachen-19-oic acid (**5**). Colourless gum, purified as its methyl ester **6** (CH_2N_2 in Et_2O), colourless gum, IR $\nu_{max}^{CCl_4}$, cm^{-1}: 3550 (OH, hydrogen bonded), 1710 (CO_2R); MS *m/z* (rel. int.): 332.235 $[M]^+$ (62) ($C_{21}H_{32}O_3$), 314 $[M-H_2O]^+$ (47), 300 $[M-MeOH]^+$ (42), 282 $[300-H_2O]^+$ (44), 275 (64), 135 (82), 119 (80), 105 (97), 93 (100). $[\alpha]_D$ positive.

Erythoxylol A-malonate (**7**). Colourless gum, purified as its methyl ester **8** (CH_2N_2 in Et_2O), colourless gum, IR $\nu_{max}^{CCl_4}$, cm^{-1}: 1760, 1740 ($OCOCH_2CO_2R$); MS *m/z* (rel. int.): 388.261 $[M]^+$ (44) ($C_{24}H_{36}O_4$), 373 $[M-Me]^+$ (2), 288 $[M-O{=}C{=}CHCO_2Me]^+$ (8), 270 $[M-HO_2CCH_2CO_2Me]^+$ (12), 257 $[M-CH_2OCOCH_2CO_2Me]^+$ (19), 148 (48), 135 (100).

$$[\alpha]_{24^\circ}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436\,nm}{+17 \quad +17.5 \quad +20 \quad +35} \quad (CHCl_3;\ c\ 1.15).$$

Nidoanomalin (**9**). Colourless gum, IR $\nu_{max}^{CCl_4}$, cm^{-1}: 1750, 1735 (CO_2R), 3040, 1645 (C=C); MS *m/z* (rel. int.): 330.256 (24) ($C_{22}H_{34}O_2$, McLafferty), 288 [330-ketone]$^+$ (11), 135 (100); CIMS (*iso*-butane) *m/z* (rel. int.): 645 $[M+1]^+$ (5), 272 $[C_{20}H_{32}]^+$ (100).

$$[\alpha]_{24^\circ}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436\,nm}{+19.7 \quad +19.7 \quad +22.1 \quad +37.6} \quad (CHCl_3;\ c\ 0.29).$$

To 5 mg **9** in 1 ml MeOH 10 mg *p*-toluene sulfonic acid was added. After 14 hr standing at room temp. TLC (Et_2O–petrol, 1:10) afforded 2 mg **3** (identical with the natural compound), 3 mg **8** (identical with the methyl ester of **7**) and 3 mg unchanged **9**.

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TWO CHROMONE GLYCOSIDES FROM *CASSIA MULTIJUGA*

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Key Word Index—*Cassia multijuga*; Leguminosae; leaves; chromone glycosides.

Abstract—Two new 2-methylchromone glycosides have been identified in the leaves of *Cassia multijuga*.

INTRODUCTION

Species of *Cassia* are rich sources of flavonoids [1, 2], anthraquinones [3, 4] and polysaccharides [5] and the plants possess important medicinal properties. All these compounds showed considerable antibiotic activity against Gram-positive organisms.

From the leaves of *Cassia multijuga*, 5 - acetonyl - 7 - hydroxy - 2 - methylchromone, 5 - acetonyl - 2 - methylchromone - 7 - *O* - β - D - glucopyranoside and

5 - acetonyl - 6 - glycosyl - 7 - hydroxy - 2 - methylchromone have been isolated and their structures elucidated. The two glycosides have not been isolated earlier from any plant source.

RESULTS AND DISCUSSION

5 - Acetonyl - 7 - hydroxy - 2 - methylchromone

$C_{13}H_{12}O_4$, mp 210° showed a characteristic blue-white fluorescence with and without ammonia. As shown by NMR it contained an acetyl group (δ 2.18 ppm) a C–Me group (δ 2.27 ppm, one non-chelated hydroxyl group (mono-acetate, mono-methyl ether; IR ν_{max} 3300 cm^{-1}), a α, β unsaturated carbonyl group (IR ν_{max} 1650 cm^{-1}) and a saturated carbonyl group (IR ν_{max} 1730 cm^{-1}, 2,4-dinitrophenyl hydrazone). Its UV spectrum was similar to those of 7-hydroxychromones or coumarins[6]. The mass fragmentation pattern, especially the elimination of the 28 and 29 *m/z* from the base peak at 190 *m/z* due to loss of a carbonyl was characteristic of a γ-pyrone system which confirmed the chromone nature of the compound. In the NMR spectrum two doublets at δ 6.68 and 6.55 and a singlet at δ 5.93 were in full agreement with a 5,7-substituted chromone derivative in which the methyl and acetonyl substituents could be placed at C-2 and C-3 respectively. Irradiation of the C-3 singlet (δ 5.93) resulted in the loss of the fine structure of the methyl signal and *vice versa* upon decoupling of the methyl protons. Similarly irradiation of the broad methylene singlet (δ 4.10) produced a sharpening of the C-6 doublet at δ 6.68. This is clear evidence that the acetonyl group is attached to C-5 and the methyl group at C-2. Hence it is 5 - acetonyl - 7 - hydroxy - 2 - methylchromone.

5 - Acetonyl - 2 - methylchromone - 7 - O - β - O - glucopyranoside

$C_{19}H_{22}O_9$ mp 260°(*d*) was a glycoside and on acid hydrolysis gave an aglycone and glucose (PC). The aglycone gave all the characteristic colour reactions of chromones and its UV spectrum was typical of 7 - hydroxychromones. It was identified as 5 - acetonyl - 7 - hydroxy - 2 - methylchromone on the basis of NMR, MS and co-chromatography. Consumption of 2 mol periodate per mol of the glycoside liberating 1 mol formic acid confirmed glucose to be in the pyranose form. The glycoside was hydrolysed by β-glucoside thereby showing the presence of a β-linkage. On the basis of these results the compound was identified as 5 - acetonyl - 2 - methylchromone 7 - *O* - β - D - glucopyranoside.

5 - Acetonyl - 6 - glycosyl - 7 - hydroxy - 2 - methylchromone

$C_{19}H_{22}O_9$ mp 190°(*d*) gave the characteristic colour reactions of chromone and was glycosidic in nature. However, neither sugar nor aglycone were obtained on acid hydrolysis. This clearly suggested that the compound is a *C*-glycoside. The *C*-glycoside nature was also demonstrated by the bands of lower intensity at 1033 and 1010 cm^{-1} in the IR[7] spectrum of the glycoside. On ferric chloride oxidation it gave glucose and a very small amount of aglycone. The compound had a UV spectrum and a diagnostic shift typical for a chromone containing a free hydroxyl group at position-7. The NMR spectrum showed signals of 5 - acetonyl - 7 - hydroxy - 2 - methylchromone and additionally those attributable to a hexosyl moiety at δ 3.35 and its anomeric proton at δ 4.55. The singlet at δ 6.55 integrated for one proton. These observations also indicated that the sugar was attached to the chromone nucleus by a C–C linkage.

The mass spectrum of the compound showed three strong peaks due to the sequential loss of three molecules of water. The intensity of the $[M-148]^+$ peak relative to $[M-149]^+$ was 42%, thus indicating that a sugar moiety must be attached to the C-6 position[8] of the chromone nucleus. The nature of the carbon-linked sugar, however, remains to be determined.

EXPERIMENTAL

Defatted leaves (5 kg) of *Cassia multijuga* were extracted with EtOH and the extract concd under red. pres. The concd extract was poured in excess cold H_2O with continuous stirring whereby a coloured residue (Fraction I) and an aq. soln (Fraction II) were obtained.

Fraction I. Solid mass was refluxed with C_6H_6, Et_2O and EtOAc. The C_6H_6 and Et_2O fractions contained mainly fatty matter and anthraquinones. The EtOAc fraction contained two compounds, one of them showing fluorescence with and without ammonia. This fraction was concd and chromatographed over Si gel and eluted with different solvents. EtOAc–MeOH (1:1) 5 - acetonyl - 7 - hydroxy - 2 - methylchromone mp 210° (EtOAc–petrol). UV λ_{max}^{EtOH} nm: 221, 243, 250 and 293; IR ν_{max}^{KBr} cm^{-1}: 3300, 1730, 1650, 1630, 1600, 1580, 1505, 1160 and 1110. NMR ($CDCl_3$, 100 MHz) δ 6.68 (1H, *d*, J = 2 Hz, C-6), 6.55 (1H, *d*, J = 2 Hz, C-8), 5.93 (1H, *br s*, C-3), 2.27 (3H, *br s*, C-Me), 4.10 (2H, *s*, CO–CH_2–), and 2.18 (3H, *s*, COMe), $[M]^+$ 232, 190 (100%) Acetate (Ac_2O–pyridine) mp 132°. Monoethyl ether (Me_2SO_4–K_2CO_3) mp 143°.

Fraction II. The aq. soln was concd under red. pres. and the concd soln extracted with different solvents of increasing polarity in a liquid–liquid extractor. The Et_2O soluble fraction contained a single entity which crystallized from EtOAc–petrol (5:2) mp 260°(*d*). UV λ_{max}^{EtOH} nm: 222, 260 and 298; IR ν_{max}^{KBr} cm^{-1}: 1730, 1650, 1630, 1590, 1575, 1500, 1160, 1110, 1050 and 730–720. NMR ($CDCl_3$, 100 MHz) δ 6.68 (1H, *d*, J = 2 Hz, C-6), 6.57 (1H, *d*, J = 2 Hz, C-8), 5.93 (1H, *br s*, C-3), 2.27 (3H, *br s*, C–Me), 4.10 (2H, *s*, CO–CH_2–), 2.18 (3H, *s*, –CO–Me), 5.00 (1H, *br s*, H-1'-glucosyl) and 3.50 (6H, *m*, glucosyl protons). Acetate (Ac_2O–MeCOONa) mp 125°.

Acid hydrolysis. 50 mg **2** was heated with 7% H_2SO_4 for 5 hr. The soln was extracted with Et_2O and the aq. layer concd and purified (PC) to give a solid which was filtered, dried in vacuum and was identical (mp, IR, UV, NMR and mass fragmentation pattern) with 5 - acetonyl - 7 - hydroxy - 2 - methylchromone.

The aq. layer was chromatographed on Whatman No. 1 paper in (a) EtOAc–pyridine–H_2O (12:5:4) and (b) EtOAc–*iso*-PrOH–H_2O (3:1:1) using pyridine with glucose, rhamnose, xylose and mixtures of these as standards. The chromatogram was developed with (a) *p*-anisidine hydrochloride (1 g) and $NaHSO_3$ (0.1 g) in MeOH (10 ml) diluted to 100 ml with *n*-BuOH and (b) aniline hydrogen oxalate spray at 120–130° for 10–16 min.

5 - Acetonyl - 6 - glycosyl - 7 - hydroxy - 2 - methylchromone. The EtOAC soluble fraction was concd and charged over Si gel and eluted with different solvents of

increasing polarity. The EtOAc eluate was found to contain a single entity which crystallized from EtOAc–petrol (3:1) mp 190°(*d*). UV λ_{max}^{EtOH} nm: 220, 245, 250, 295; IR ν_{max}^{KBr} cm^{-1}: 3300, 1740, 1655, 1635, 1590, 1575, 1500, 1155, 1033, 1010 and 730–720. NMR (DMSO-d_6, 100 MHz) δ 6.55 (1H, *s*, C-8), 5.93 (1H, *br s*, C-3), 2.27 (3H, *br*, C–Me), 4.10 (2H, *s*, CO–CH_2–), 2.18 (2H, *s*, COMe), 3.55 (6H, *m*, glucosyl), 4.55 (1H, *d*, J = 9 Hz, H-1 of glucosyl).

Oxidation of glycoside with ferric chloride. The glycoside (50 mg), $FeCl_3$ (100 mg), conc HCl (4 ml) and conc H_2SO_4 (2 drops) were heated at 95° for 20 hr. A black solid was filtered from the soln, dried and extracted with boiling toluene. Removal of toluene yielded the aglycone, 5 - acetonyl - 7 - hydroxy - 2 - methylchromone.

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3, 5, 4′-TRIHYDROXY-6, 7-DIMETHOXYFLAVONE 3-GLUCOSIDE FROM *SESUVIUM PORTULACASTRUM*

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Key Word Index—*Sesuvium portulacastrum*; Aizoaceae; 3,5,4′-trihydroxy-6,7-dimethoxyflavone 3-*O*-glucoside; flavonol glycoside; eupalitin; eupalitin 3-*O*-glucoside.

Abstract—A new flavonol glycoside 3,5,4′-trihydroxy-6,7-dimethoxyflavone 3-*O*-glucoside has been characterized from *Sesuvium portulacastrum*, together with its aglycone, eupalitin.

Earlier workers have reported [1] the occurrence of eupalitin 3-*O*-rutinoside and α- and β-ecdysones from *Sesuvium portulacastrum*. In the present investigation of the same plant a second glycoside (**1**) was isolated as yellow crystals, m.p. 223° and shown to be a flavonol with free hydroxyls at C–5 and C–4′ and a substituted hydroxyl at C–7 from the UV data. The ^{1}H NMR spectrum (60 MHz) in DMSO-d_6 showed doublets (J = 10 Hz) at δ 6.8 and 8.0 representing A_2B_2 system in ring C. A singlet at δ 6.67 was assigned to *C*–8 proton. The presence of two methoxyls was inferred by signals at δ 3.8 and 3.85. Signals for free phenolic hydroxyls appear at δ 9.03 and 9.73. IR ν_{max}^{KBr} cm^{-1}: 3400, 1660, 1620, 1120, 1060. Strong absorption at 1060 and 1120 cm^{-1} reflected the glycosidic nature of the compound [2]. The mass spectrum of the compound was similar to that of the aglycone eupalitin ([M]$^+$ 330) owing to the easy elimination of the sugar moiety from the glycoside [3]. The position of the sugar moiety in **1** was ascertained by methylation with dimethyl sulphate followed by hydrolysis (7% methanolic hydrochloric acid) which yielded 3-hydroxy-5, 6, 7, 4′-tetramethoxyflavone (**2**, mp 123°, yellow needles), whereas methylation with diazomethane and subsequent hydrolysis produced 3, 5-dihydroxy-6, 7, 4′-trimethoxyflavone (**3**, mikanin mp 222°). Hydrolysis of **1** (7% methanolic hydrochloric acid) yielded an aglycone which was identified as 3, 5, 4′-trihydroxy-6, 7-dimethoxyflavone (**4**, eupalitin) by UV data and ^{1}H NMR studies. The sugar moiety was determined as glucose by co-chromatography on paper with an authentic sample (R_f 0.20, descending, *n*-BuOH–HOAc–H_2O, 4:1:5; aniline hydrogen phthalate spray). From these data **1** is characterized as the new glycoside 3, 5, 4′-trihydroxy-6, 7-dimethoxyflavone 3-*O*-glucoside. This structure was confirmed by synthesis of 3-glucosyloxy-5, 4′-dihydroxy-6, 7-dimethoxyflavone by treating the