# 58. Boscialin and Boscialin 4′-*O*-Glucoside, Two New Compounds Isolated from the Leaves of *Boscia salicifolia* OLIV.

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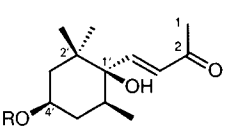
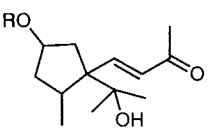
Boscialin (**1**), a new compound structurally related to the ionones and abscisic acid, and its 4′-*O*-glucoside **2** were isolated from the MeOH extract of the leaves of the African medicinal plant *Boscia salicifolia*. The structures of the two compounds were determined mainly by NMR spectroscopy and by acid hydrolysis of the glycoside.

**1. Introduction.** – *Boscia salicifolia* OLIV. (Capparidaceae) is a common tree which grows to 5–6 m height in Tanzania. Its mashed leaves or the juice obtained from the leaves are used in traditional medicine to aid wound-healing processes [3]. In the course of our investigation of the constituents of this plant [2] [4] [5], two new compounds, boscialin (**1**) and its 4′-*O*-glucoside **2**, were isolated from the MeOH extract of the dried leaves.

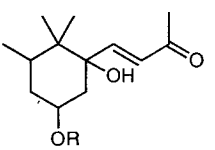
The dried leaves of *Boscia salicifolia* were powdered and then extracted exhaustively in succession with petroleum ether, $CHCl_3$, MeOH, and MeOH/$H_2O$. The MeOH extract



**1** R = H, Boscialin

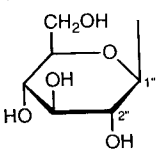


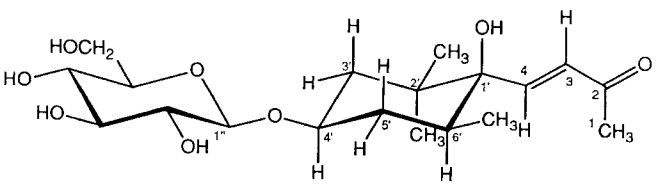
**3**



**4**

**2** R =





**5**

[1]) Part of the diploma thesis of *N.P.* [1].

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was fractionated by droplet counter-current chromatography (DCCC); its constituents were then isolated and purified by column chromatography. Besides four flavonol glycosides [5], a new compound **1**, structurally related to the ionones and abscisic acid (= 5-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-enyl)-3-methylpenta-2,4-dienoic acid), was obtained together with its 4′-*O*-glucoside **2.**

**2. Stucture Elucidation.** – Compound **2** was readily recognized as a glycoside. In the FAB-MS, 180 amu were lost from the molecular ion, which pointed to the possible loss of a hexose. Furthermore, eight signals of $sp^3$-C-atoms bound to an O-atom were clearly visible in the $^{13}$C-NMR spectrum including one C-atom involved in a acetal function ($\delta$ *ca.* 100 ppm). Acid hydrolysis of **2** gave compound **1** as the aglycone and a sugar which was identified as glucose by TLC comparison with authentic samples of L-arabinose, D-xylose, D-galactose, D-glucose, D-mannose, D-fructose, and L-rhamnose. The $^{13}$C-NMR spectrum of this sugar was identical with that of an authentic sample of D-glucose.

The $^{13}$C-NMR chemical shifts of the glucose moiety of **2** were in excellent agreement with those obtained for other $\beta$-D-glucopyranosides [5] [6] and thus proved that the sugar moiety in **2** was present as a $\beta$-D-pyranoside. The $\beta$-D-configuration of the glycosidic linkage was corroborated by the coupling constant of 8 Hz observed for the anomeric proton [7].

Most of the constitution of the aglycone moiety was established from the $^1$H-NMR spectrum and extensive H,H-spin decoupling experiments: an (*E*)-configurated disubstituted double bond, an acetyl fragment, two Me groups attached to a quaternary C-atom and an aliphatic fragment $CH_2$–CH(O)–$CH_2$–$CH(CH_3)$. The assignments of the H- and C-resonances were completed and corroborated by a C,H-shift-correlated 2D-NMR spectrum (HETCOR). The structural elements recognized so far could still be assembled in several possible ways. However, only the constitution shown in formula **2** was consistent with the set of C,H long-range couplings observed in two C,H-shift-correlated 2D-NMR spectra which were optimized for $J$(C,H) = 7 and 10 Hz, respectively (see *Exper. Part*).

Constitutions with five-membered rings such as, *e.g.*, **3** could be ruled out, since the protons of both Me groups at C(2′) showed a long range coupling with C(3′). In constitution **3** or similar ones, where the two Me groups are not directly attached to the ring, the corresponding coupling would involve four bonds and, therefore, hardly be observable. Similar argumentation ruled out compound **4** where the secondary Me group has a different position than in **2.** The protons of this Me group should exhibit a three-bond coupling with the quaternary C-atom bearing the other two Me groups. No cross-peak could, however, be detected for such an interaction. In **2**, the coupling pathway extends over four bonds which is in accord with the absence of the respective cross-peak.

Information about the relative configurations and the conformation in the aglycone part of **2** was obtained from the coupling constants observed for the ring protons and from homonuclear NOE experiments (see *Exper. Part*). Whereas the relative configurations of the three chiral centers of **2** have thus been determined, it was not possible from our studies to determine the absolute configuration of the aglycone.

One of the protons at C(5′) of **2** appears as a *q* with $J$ = 12.5 Hz. This has to be an axial proton with only geminal and axial vicinal neighbors. This means that H–C(6′) and H–C(4′) have to be in axial positions, too. Similarly, the signal of the axial proton at C(3′) is readily recognized, as it is a *t* with $J$ = 12 Hz. The strong mutual NOE's observed between H–C(4′), H–C(6′), and one of the Me–C(2′) corroborates their spatial proximity and thus their respective *cis*-arrangement and axial position. In a similar way, H–C(4) is related to H–C(6′) and the axial Me–C(2′) through substantial NOE's. This is only possible when the butenone chain at C(1′) is in the equatorial position and the conformation along the C(4)–C(1′) bond is as shown in formula **5.** A typical W-long-range coupling over four bonds connects the equatorial H–C(3′) and H–C(5′) and confirms their assignments.

**3. Discussion.** – Boscialin (**1**) and its glucoside **2** are compounds which are constitutionally related to β-ionone and abscisic acid. Several substances which closely resemble boscialin have been reported in the past. Synthetic compounds include, *e.g.*, one of the photoproducts of a β-ionone derivative [8]. A compound with the same substitution pattern in the cyclohexane part as **2** but with opposite relative configuration at C(6′) was obtained in the course of studies concerning the synthesis of acetylenic carotenoids [9]. Boscialin (**1**) can be regarded as a reduced analog of dehydrovomifoliol, a natural product isolated as one of the metabolites when α-ionone was fed to the fungus *Cercospora rosicola* [10]; dehydrovomifolid is also a constituent of several plants, including the grapes of *Vitis vinifera* L. [11]. The secondary OH group available at C(4′) of **1** seems to be preferred for glycosylation in the plant as demonstrated by the constitution of **2**. In abscisic acid, which only contains the tertiary OH–C(1′), glycosylation of this sterically rather hindered group [12] is often avoided by formation of a glycosyl ester [13].

Although we have no explanation for the phenomenon, it is interesting to note that in the two batches of leaves which were collected during different seasons of the year, the predominant forms in which boscialin occurs are different. The leaves harvested in June contained mostly the aglycone **1**, whereas in those collected in October, only traces of **1** could be detected, and the predominant form was the glycoside **2**. A similar seasonal dependence of the ratio between aglycone and glycoside was recently reported for the plant-growth regulator abscisic acid and its glucosyl ester in grape leaves [14].

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## Experimental Part

1. *General.* Droplet counter-current chromatography (DCCC [15]): *Büchi-670*-DCC-chromatograph equipped with 294 tubes (40 cm, i.d. 2.7 mm). Reversed-phase chromatography: *Lobar RP-8* column (40–63 μm; 24 cm × 10 mm; *Merck*) equipped with a *Duramat 80* pump (*Chemie und Filter*, Regensdorf). Normal-phase column chromatography: silica gel *60* (40–63 μm; *Chemische Fabrik Uetikon*). TLC: silica gel precoated Al sheets (*Merck*) with AcOEt/MeOH/$H_2O$ 100:13.5:10 (system I) for general screening, UV (254 nm) detection or with vanillin/$H_2SO_4$ [16]; for sugars with AcOEt/MeOH/$H_2O$/AcOH 13:3:3:4 (system II), acetone/3% boric acid in $H_2O$ 3:1 (system III), $CHCl_3$/MeOH/acetone/$H_2O$ 3:3:3:1 (system IV), detection with naphthoresorcinol [17]. $^1$H-NMR and $^{13}$C-NMR: *Varian VXR-400* at 400 and 101 MHz, respectively (*K. Aegerter*, Dr. *H. Nadig*, *K. Ulrich*); chemical shifts in δ (ppm) relative to internal TMS ( = 0 ppm), coupling constants *J* in Hz; $^{13}$C-NMR multiplicities by APT experiments. FAB-MS: *VG 70-250* (Dr. *H. Nadig*).

2. *Plant Material.* Leaves of *Boscia salicifolia* OLIV. were collected east of Ifakara, Kilombero District, Morogoro Region, Tanzania, in June 1984 (first batch) and in October 1986 (second batch). The plant material was identified by Dr. *F. Haerdi* (*F. Hoffmann-La Roche AG*, Basel, and Pharmazeutisches Institut der Universität Basel); a voucher specimen was deposited with the Botanisches Institut der Universität Basel.

3. *Extraction and Isolation.* The dried leaves (100 g from the first batch, 150 g from the second) were ground to a fine powder and extracted at r.t. successively with petroleum ether (3 × 1l), $CHCl_3$ (3 × 1l), and MeOH (3 × 1l). The MeOH extracts were evaporated to give 12.4 and 31.6 g of syrupy material, respectively. Portions of 4–5 g each of this material were fractionated by DCCC ($CHCl_3$/MeOH/$H_2O$/i-PrOH 5:6:4:1, ascending mode, flow rate *ca.* 30 ml/h; monitoring by TLC, system I) to give *ca.* 150 fractions.

*Fr. 110–116* of one of the DCCC runs from the first batch of leaves (25.1 mg) were then successively purified by low-pressure liquid chromatography (LPLC) on a *Lobar RP-8* column using MeOH/$H_2O$ 4:6 (4.8 mg of **1** in *Fr. 4–6*) and normal-phase chromatography on silica gel *60* using $CHCl_3$/AcOEt 1:1: 2.3 mg of **1**. From three DCCC runs with the MeOH extract from the second batch of leaves, the fractions containing the substance with an $R_f$ value of 0.4 on TLC (system I) were pooled and evaporated. The residue (511 mg) was subjected to LPLC on a *Lobar RP-8* column with MeOH/$H_2O$ 1:1 to give 48 mg of crude **2**, which were rechromatographed on silica gel *60* with $CH_2Cl_2$/MeOH 17:3: 16 mg of **2**.

*Data of Boscialin* (= (E)-*4-[(1'R*,4'R*,6'S*)-1',4'-Dihydroxy-2',2',6'-trimethylcyclohexyl]but-3-en-2-one*; **1**): Almost colorless, amorphous solid. TLC ($SiO_2$, system I): $R_f$ 0.64, red with vanillin/$H_2SO_4$. $[\alpha]_D^{23} = -19\pm5$ ($c = 0.14$, $CHCl_3$). $^1$H-NMR (400 MHz, $CD_3OD$): 6.88 (*d*, $J = 16$, H–C(4)); 6.34 (*d*, $J = 16$, H–C(3)); 3.83 (*m*, H–C(4')); 2.28 (*s*, $CH_3$(1)); 2.10 (*m*, H–C(6')); 1.7, 1.4 (2*m*, 2 H each, $CH_2$(5'), $CH_2$(3')); 1.04 (*s*, $CH_{3ax}$–C(2')); 0.86 (*s*, $CH_{3eq}$–C(2')); 0.81 (*d*, $J = 6.5$, $CH_3$–C(6')). $^1$H-NMR (400 MHz, $CDCl_3$): 6.75 (*d*, $J = 16$, H–C(4)); 6.37 (*d*, $J = 16$, H–C(3)); 3.89 (*m*, H–C(4')); 2.28 (*s*, $CH_3$(1)); 2.08 (*m*, H–C(6')); 1.83 (br. *dt*, $J = 12.5, 4$, $H_{eq}$–C(5')); 1.62 (*t*, $J = 12$, $H_{ax}$–C(3')); *ca.* 1.55 ($H_{eq}$–C(3'), under $H_2O$ signal); 1.34 (*q*, $J = 11.5$, $H_{ax}$–C(5')); 1.04 (*s*, $CH_{3ax}$–C(2')); 0.88 (*s*, $CH_{3eq}$–C(2')); 0.81 (*d*, $J = 6.5$, $CH_3$–C(6')). $^{13}$C-NMR (101 MHz, $CD_3OD$): 200.8 (*s*, C(2)); 154.4 (*d*, C(4)); 131.6 (*d*, C(3)); 78.9 (*s*, C(1')); 67.3 (*d*, C(4')); 45.8 (*t*, C(3')); 41.0 (*s*, C(2')); 39.6 (*t*, C(5')); 35.3 (*d*, C(6')); 27.4 (*q*, C(1)); 26.0 (*q*, $CH_{3eq}$–C(2')); 25.2 (*q*, $CH_{3ax}$–C(2')); 16.5 (*q*, $CH_3$–C(6')). $^{13}$C-NMR (101 MHz, $CDCl_3$): 197.5 (C(2)); 150.5 (C(4)); 130.3 (C(3)); *ca.* 77 (C(1'), under the solvent signal); 66.4 (C(4')); 45.1 (C(3')); 39.9 (C(2')); 39.0 (C(5')); 34.0 (C(6')); 28.2 (C(1)); 25.2 ($CH_3$–C(2')); 24.6 ($CH_3$–C(2')); 15.9 ($CH_3$–C(6')). EI-MS: 208 (6.4, $[M - H_2O]^+$), 170 (24), 126 (43), 111 (100), 98 (21), 83 (14), 71 (22), 55 (32), 43 (92). CI-MS ($NH_3$): 244 (0.71, $[M + NH_4]^+$), 227 (2.3, $[M + H]^+$), 209 (100, $[M + H - H_2O]^+$), 170 (13), 126 (8.0), 111 (10). FAB-MS (pos. mode, glycerol): 453 (4.7, $[M_2 + H]^+$), 421 (3.5), 399 (23), 319 (3.3, $[M + Gly + H]^+$), 299 (3.6), 249 (3.8, $[M + Na]^+$), 227 (8.4, $[M + H]^+$), 209 (72, $[M + H - H_2O]^+$), 197 (17), 153 (25), 109 (27), 43 (100). FAB-MS (neg. mode, glycerol): 225 (100, $[M - H]^-$).

*Data of Boscialin 4'-*O*-Glucoside* (= (E)-*4-[(1'R*,4'R*,6'S*)-4'-[(β-*D*-Glucopyranosyl)oxy]-1'-hydroxy-2',2',6'-trimethylcyclohexyl]but-3-en-2-one*; **2**): Almost colorless amorphous solid. TLC ($SiO_2$, system I): $R_f$ 0.40, red-violet with vanillin/$H_2SO_4$. $[\alpha]_D^{22} = -27\pm5$ ($c = 0.40$, MeOH). $^1$H-NMR (400 MHz, $CD_3OD$): 6.88 (*d*, $J = 16$, H–C(4)); 6.34 (*d*, $J = 16$, H–C(3)); 4.36 (*d*, $J = 8$, H–C(1'')); 3.98 (*ddt*, $J = 11.5$, 11, 4, H–C(4')); 3.87 (br. *d*, $J = 12$, 1 H, H–C(6'')); 3.66 (*ddd*, $J = 11.5$, 4, 1.5, 1 H, H–C(6'')); 3.33–3.24 (*m*, H–C(3''), H–C(4''), H–C(5'')); 3.14 (*dd*, $J = 9$, 8, H–C(2'')); 2.28 (*s*, $CH_3$(1)); 2.12 (*dqd*, $J = 12$, 6.5, 4, H–C(6')); 1.86 (*dtd*, $J = 12.5$, 4, 2, $H_{eq}$–C(5')); 1.70 (*t*, $J = 12$, $H_{ax}$–C(3')); 1.60 (*ddd*, $J = 13$, 4.5, 2, $H_{eq}$–C(3')); 1.51 (*q*, $J = 12.5$, $H_{ax}$–C(5')); 1.05 (*s*, $CH_{3ax}$–C(2')); 0.88 (*s*, $CH_{3eq}$–C(2')); 0.81 (*d*, $J = 6.5$, $CH_3$–C(6')). NOE's (irradiated H→ affected H; ++ = strong, + = medium, (+) = weak): H–C(3)→H–C(1) (++), $CH_{3eq}$–C(2') ((+)), $CH_3$–C(6') ((+)); H–C(4) → H–C(1) (++), $CH_{3ax}$–C(2'), (+), H–C(6') (+); $CH_{3ax}$–C(2') →H–C(4) (++), $CH_{3eq}$–C(2') ((+)), $H_{eq}$–C(3') ((+)), H–C(4') (++), H–C(6') (+); $CH_{3eq}$–C(2') →H–C(3) (++), $CH_{3ax}$–C(2') ((+)), $H_{ax}$–C(3') ((+)), $H_{eq}$–C(3') ((+)); H–C(4')→$CH_{3ax}$–C(2') ((+)), $H_{eq}$–C(5') ((+)), H–C(6') (+), H–C(1'') (++); H–C(6')→H–C(3) (+), H–C(4) (++), $CH_{3ax}$–C(2') ((+)), H–C(4') (++), $H_{eq}$–C(5') ((+)); $CH_3$–C(6')→H–C(3) (++), $H_{ax}$–C(5') ((+)), $H_{eq}$–C(5') ((+)), H–C(6') (+). $^{13}$C-NMR (101 MHz, $CD_3OD$): 200.8 (*s*, C(2)); 154.3 (*d*, C(4)); 131.6 (*d*, C(3)); 102.8 (*d*, C(1'')); 79.0 (*s*, C(1')); 78.1 (*d*, C(3'') or C(5'')); 77.9 (*d*, C(5'') or C(3'')); 75.5 (*d*, C(4')); 75.2 (*d*, C(2'')); 71.8 (*d*, C(4'')); 62.9 (*t*, C(6'')); 42.5 (*t*, C(3')); 41.0 (*s*, C(2')); 37.9 (*t*, C(5')); 35.4 (*q*, C(6')); 27.4 (*q*, C(1)); 26.0 (*q*, $CH_3$–C(2')); 25.1 (*q*, $CH_3$–C(2')); 16.5 (*q*, $CH_3$–C(6')). C,H-Shift correlation (optimized for $J$(C,H) = 7 Hz): C(2)/*H*–C(1), *H*–C(4); C(3)/*H*–C(1); C(1')/$CH_{3ax}$–C(2'), $CH_{3eq}$–C(2'); C(2')/$CH_{3ax}$–C(2'), $CH_{3eq}$–C(2'), $H_{ax}$–C(3'); $CH_{3ax}$–C(2')/$CH_{3eq}$–C(2'), $H_{ax}$–C(3'); $CH_{3eq}$–C(2')/$CH_{3ax}$–C(2'); C(3')/$CH_{3ax}$–C(2'), $CH_{3eq}$–C(2'); C(4')/$H_{ax}$–C(3'), $H_{ax}$–C(5'); C(5')/$CH_3$–C(6'); C(6')/$H_{ax}$–C(5'), $CH_3$–C(6'). C,H-Shift correlation (optimized for $J$(C,H) = 10 Hz): C(2)/*H*–C(1), *H*–C(4); C(1')/*H*–C(4), $CH_{3ax}$–C(2'), $CH_{3eq}$–C(2'), $H_{eq}$–C(3'); C(2')/$CH_{3ax}$–C(2'), $CH_{3eq}$–C(2'); $CH_{3ax}$–C(2')/$CH_{3eq}$–C(2'), $H_{ax}$–C(3'); $CH_{3eq}$–C(2')/$CH_{3ax}$–C(2'); C(3')/$CH_{3ax}$–C(2'), $CH_{3eq}$–C(2'); C(6')/$H_{ax}$–C(5'). FAB-MS (pos. mode, glycerol): 411 (3.1, $[M + Na]^+$), 389 (4.7, $[M + H]^+$), 387 (4.7), 371 (8.3, $[M + H - H_2O]^+$), 209 (99, $[M + H - glucose]^+$), 43 (100, $[CH_3CO]^+$). FAB-MS (pos. mode, glycerol + NaCl): 469 (23, $[M + Na + NaCl]^+$), 433 (16, $[M + 2Na - H]^+$), 411 (100, $[M + Na]^+$), 409 (37), 209 (22, $[M + H - glucose]^+$), 43 (81, $[CH_3CO]^+$).

4. *Acid Hydrolysis.* The glucoside **2** (4 mg) was refluxed with 2N HCl (4 ml) for 1 h. The mixture was then extracted with AcOEt. The product obtained from the AcOEt layer showed the same properties as **1** (TLC, $^1$H-NMR). The sugar obtained from the aq. layer was identified as glucose by TLC ($SiO_2$, systems II, III, and IV) with authentical references. Its $^{13}$C-NMR spectrum was identical with that of glucose. $[\alpha]_D$: positive ($H_2O$).

## REFERENCES


[1] N. Pauli, Diplomarbeit, Universität Basel, 1989.
[2] A. Walter, Dissertation, Universität Basel, 1989.
[3] F. Haerdi, *Acta Tropica Suppl.* **1964**, *8*, 1.
[4] A. Walter, U. Séquin, *Chimia* **1989**, *43*, 353.
[5] A. Walter, U. Séquin, *Phytochemistry* **1990**, *29*, in press.
[6] K.R. Markham, V.M. Chari, in 'The Flavonoids: Advances in Research', Eds. J.B. Harborne and T.J. Mabry, Chapman and Hall, London–New York, 1982, p. 19–134; K.R. Markham, B. Ternai, R. Stanley, H. Geiger, T.J. Mabry, *Tetrahedron* **1978**, *34*, 1389; P.A.J. Gorin, M. Mazurek, *Can. J. Chem.* **1974**, *53*, 1212; R.G.S. Ritchie, N. Cyr, B. Korsch, H.J. Koch, A.S. Perlin, *ibid.* **1974**, *53*, 1424.
[7] H. Günther, 'NMR-Spektroskopie', 2. Aufl., Georg Thieme Verlag, Stuttgart, 1983, p. 105.
[8] K. Murato, H.R. Wolf, O. Jeger, *Helv. Chim. Acta* **1980**, *63*, 2212.
[9] A.J. Davies, A. Khare, A.K. Mallams, R.A. Massy-Westropp, G.P. Moss, B.C.L. Weedon, *J. Chem. Soc., Perkin Trans. 1* **1984**, 2147.
[10] S.M. Norman, S.M. Poling, V.P. Maier, M.D. Nelson, *Agric. Biol. Chem.* **1985**, *49*, 2887.
[11] C.R. Strauss, B. Wilson, P.J. Williams, *Phytochemistry* **1987**, *26*, 1995.
[12] B.R. Loveys, B.V. Millborrow, *Aust. J. Plant. Physiol.* **1981**, *8*, 571.
[13] S.M. Southwick, A. Chung, T.L. Davenport, J.W. Ryan, *Plant Physiol.* **1986**, *81*, 323.
[14] M. Broquedis, T. Koussa, J. Bouard, *Connaiss. Vigne Vin* **1988**, *22*, 295.
[15] K. Hostettmann, *Planta Med.* **1980**, *39*, 1; K. Hostettmann, M. Hostettmann, A. Marston, 'Preparative Chromatography Techniques', Springer Verlag, Berlin–Heidelberg–New York, 1986, p. 80–101.
[16] H. Wagner, S. Bladt, E.M. Zgainski, 'Drogenanalyse', Springer-Verlag, Berlin–Heidelberg–New York, 1983, p. 164.
[17] 'Anfärbereagenzien für Dünnschicht- und Papierchromatographie', E. Merck, Darmstadt, 1980.