Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 66 (2005) 1698–1706
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Cyclohexanoid protoflavanones from the stem-bark and roots of *Ongokea gore* ☆

Gerold Jerz [a,*], Reiner Waibel [b], Hans Achenbach [b]

[a] *Institute of Food Chemistry, Technical University of Braunschweig, Schleinitz-Strasse 20, D-38106 Braunschweig, Germany*
[b] *Institute of Pharmacy and Food Chemistry, University of Erlangen-Nürnberg, Schuh-Strasse 19, D-91052 Erlangen, Germany*

Received 14 February 2005; received in revised form 14 April 2005
Available online 26 May 2005

Abstract

Phytochemical investigation of root and stem-bark of the West African medicinal plant *Ongokea gore* resulted in the isolation of four novel flavonoids with an unusual cyclohexyl substituent instead of the common aromatic ring B. The structures of the isolated compounds were elucidated by spectroscopic methods, mainly 1D and 2D NMR, and subsequently, the structures were corroborated by chemical conversion to (−)-(*S*)-sakuranetin. The absolute configurations, and preferred conformations were determined by NOE experiments and CD measurements.

Keywords: *Ongokea gore*; Olacaceae; Cyclohexanoid protoflavanones; Ongokein; (2*S*)-Ongokein-4′-one; (2*S*)-4′,4′-Dimethoxy-ongokein; (2*S*)-*cis*-4′-Hydroxy-ongokein; (2*S*)-*trans*-4′-Hydroxy-ongokein; (2*S*)-Ongokein-2′-en-4′-one; (2*S*)-5-Hydroxy-2-(4′-oxo-2′-cyclohexenyl)-7-methoxychroman-4-one; (−)-(*S*)-Sakuranetin

1. Introduction

In the course of our phytochemical investigations of tropical medicinal plants (Waibel et al., 2003) we recently studied extracts of *Ongokea gore* Engler (Olacaceae), a tropical tree indigenous to river forests of West- and Central Africa which grows up to 30 m high. In ethnomedicine of Ghana, the seed oil of *O. gore*, derived from the, 'boleko-nut', and also prepared decocts of the stem-bark are used as a purgative. The sap of the bark is used to treat bleedings (Irvine, 1961; Hegnauer, 1990). Previous phytochemical investigations were mainly focussed on the chemical composition of the *Ongokea* seed oil ('boleko'-, 'isano'-oil), and resulted in the isolation of unusual conjugated C_{18}-acetylenic fatty acids, specific chemotaxonomic markers of the plant families Olacaceae and Santalaceae (Gunstone and Sealy, 1963; Miller et al., 1977).

All investigated extracts of *O. gore* showed significant cytotoxic activities in the 'brine shrimp'-assay (*Artemia salina*) (Meyer et al., 1982). In the course of our attempts to find the active principle, we isolated from stem-bark and roots of *O. gore* four novel flavanones, all containing the biosynthetically unusual 1′-hydroxycyclohexyl B-ring moiety. Their structure elucidation will be described here.

Flavonoids with a non-aromatic B-ring sometimes named protoflavonoids, represent a class of rare natural products. Hitherto they are solely known as constituents from fern varieties, i.e., protofarrerol, and dihydroprotofarrerol from *Leptorumohra miqueliana* (Aspidiaceae) (Noro et al., 1969; Fukushima et al., 1969), protofarrerol 7-*O*-β-D-glucoside *Monachosorum henryi* CHRIST (Pteridaceae) (Murakami et al., 1987), the 4′-*O*-β-glucoside

☆ Part 93 in the series "Constituents of Tropical Medicinal Plants". For part 92 see Waibel et al. (2003).

* Corresponding author. Tel.: +49 531 391 7208; fax: +49 531 391 7230.
E-mail address: g.jerz@tu-bs.de (G. Jerz).

0031-9422/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2005.04.031

of protogenkwanin *Equisetum arvense* L. (Equisetaceae) (Hauteville et al., 1980, 1981), also dihydroprotogenkwanin (Adam, 1999), and protogenkwanone, tetrahydroprotogenkwanone, tetrahydroprotogenkwanin from *Pseudophegopteris* species (Thelypteridaceae) (Wada et al., 1987). This is the first report on the occurrence of protoflavonoids in plants of the *Spermatophytae* group, a finding which might be of potential chemotaxonomic significance.

The protoflavanones of *O. gore* were named (2*S*)-ongokein-4′-one (**1**), (2*S*)-4′,4′-dimethoxy-ongokein (**2**), (2*S*)-*cis*-4′-hydroxy-ongokein (**3**), and (2*S*)-*trans*-4′-hydroxy-ongokein (**4**).

2. Results and discussion

2.1. Isolation of protoflavanones

Root and stem-bark of *O. gore* were freeze-dried, milled to coarse powder, defatted with petrol, and then repeatedly macerated with methanol. The combined polar extracts were diluted with water, and re-extracted with dichloromethane. The dichloromethane extract mainly consisted of free C_{18}-acetylenic fatty acids which were already described as principal components of the, *isano* seed oil of *O. gore* (Gunstone and Sealy, 1963; Miller et al., 1977). Size exclusion chromatography (Sephadex LH-20) of the polar extract separated a fraction of compounds with high affinity to the gel material. Further separation of this fraction by CC (silica gel) and final purification by prep. RP-HPLC yielded compounds **1**–**4**.

2.2. (2S)-Ongokein-4′-one (1)

The ^{1}H NMR spectrum of **1** exhibited typical properties of a flavanone. Doublets of two meta coupled aromatic protons, the singlet of a methoxy group, and a significantly downfield shifted singlet (H/D exchangeable) attributable to a phenolic proton bound in a six-ring chelat complex of a flavanone partial structure, as well as three signals with the typical ABX coupling pattern of the C-ring could be observed. However, all resonances of a typical aromatic flavonoid B-ring were completely missing. Instead, the complex resonances of eight aliphatic protons appeared, suggesting the presence of a hydrogenated B-ring moiety. This was confirmed by HMQC and HMBC experiments (Fig. 1), which also allowed the complete assignment of all ^{1}H and ^{13}C resonances.

The ^{13}C NMR spectroscopic data (Table 1) are partially in agreement with reference data of protoflavon tetrahydroprotogenkwanin – which is missing a chiral C-2 position (Murakami et al., 1987).

Circular dichroism spectroscopy (CD) established 2*S*-chirality in **1** (Fig. 2): the observed positive Cotton effect at λ 325 nm, and a negative at λ 270 nm are in good

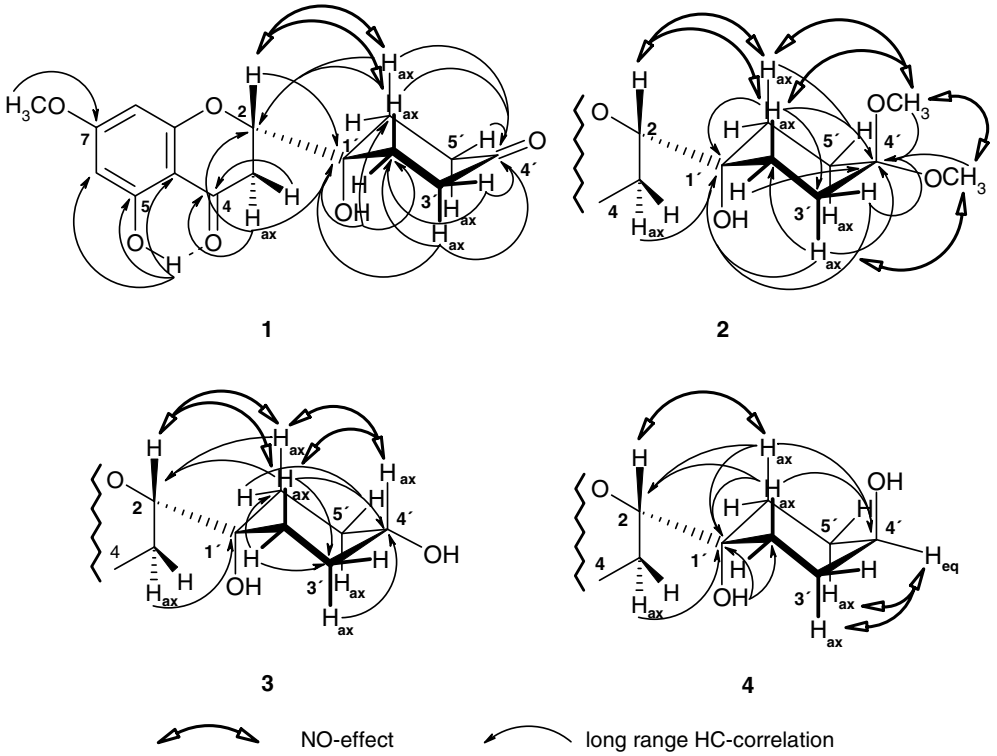

Fig. 1. Important $^{2,3}J$-CH–^{1}H{^{13}C} correlations of the cyclohexanoid flavonoids **1**–**4**, and conformational relevant NO-effects (in $CDCl_3$) confirming the preferred $_{1'}C^{4'}$-chair-conformation.

Table 1
^{13}C NMR spectral data of **1**–**4**, **5a/5b**, and **6**–**8** in $CDCl_3$: δ [ppm]

Carbon	1	2	3	4	5a/5b[c]	6	7	8
2	82.7	83.3	83.3	82.8	78.4/79.0	78.9	83.3	83.3
3	36.4	36.3	36.3	36.0	40.2	43.2	37.9	37.8
4	195.7	196.4	196.4	196.6	198.5/198.4	196.0	189.1	189.3
5	164.1	164.0	164.0	163.9	162.2/162.4	162.9	151.8	151.8
6	95.2	95.0	95.0	94.9	94.15/94.20	94.2	104.7	104.6
7	167.9	167.8	167.8	167.8	168.03/168.05	168.0	169.5	169.5
8	94.2	94.0	94.0	94.0	95.2	95.1	99.8	99.4
9	161.9	162.3	162.3	162.4	164.15/164.20	164.1	163.6	163.4
10	102.8	102.9	102.9	102.9	103.0/103.05	103.1	107.9	107.9
1′	70.9	71.4	70.8	71.7	38.75/39.30	130.7	70.9	71.5
2′	31.9[a]	28.7[a]	30.4[a]	26.5[a]	148.0/148.5	128.0	30.2[a]	26.8[a]
3′	36.1[b]	27.3[b]	30.0[b]	28.2[b]	130.85/131.50	115.7	26.2[b]	25.0[b]
4′	210.6	99.5	70.0	65.8	195.1/195.2	156.1	71.6	69.0
5′	36.2[b]	27.3[b]	30.1[b]	28.2[b]	36.55/36.70	115.7	26.3[b]	25.0[b]
6′	33.7[a]	30.8[a]	32.0[a]	28.0[a]	23.7/24.8	128.0	31.9[a]	28.9[a]
–OCH$_3$	55.7	55.6	55.7	55.6	55.7	55.7	55.8	55.8
–OCH$_3$		47.70						
–OCH$_3$		47.65						
–*C*OCH$_3$							165.4 **at C-5**	165.4 **at C-5**
–*C*OCH$_3$							170.6 **at C-4′**	170.5 **at C-4′**
–CO*C*H$_3$							21.1	21.0
–CO*C*H$_3$							21.4	21.4

[a–b] Assignments in a column with same superscript might be interchangeable.
[c] Epimeric mixture of **5a/5b**.

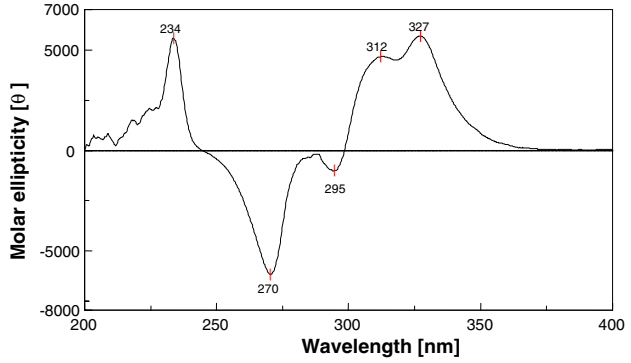

Fig. 2. CD-curve of protoflavanone **1** (in MeOH) indicating the (2*S*)-configuration.

agreement with reference data of (2*S*)-configured flavanones such as (−)-(*S*)-naringenin (Gaffield, 1970), and the protoflavonoid (2*S*)-protofarrerol (Fukushima et al., 1969). Knowing the 2*S*-configuration, results of NOE experiments (Fig. 1) elucidated the cyclohexanoid B ring being in a predominant $_{1'}C^{4'}$-conformation with the *tert*-OH group at C-1′ in the energetically most favourable axial orientation. There are no other stereochemical configurations which are eligible for the observed NO effects.

The free rotational flexibility of the cyclohexanone ring along the C-2/C-1′ bond is demonstrated by strong NOEs between H-2 and H-2′$_{ax}$, H-6′$_{ax}$ and the proton of the *tert*-OH group.

In accordance to the general flavonoid fragmentation pathway (Audier, 1966), the EI-MS of **1** showed key-fragments at *m*/*z* 166 and *m*/*z* 193 for the methoxylated A-ring and the chromanone partial structure, respectively. Signals of lower abundance at *m*/*z* 113, and *m*/*z* 95 were related to the cyclohexanone moiety.

2.3. (2*S*)-4′,4′-Dimethoxy-ongokein (**2**), (2*S*)-cis-4′-hydroxy-ongokein (**3**), (2*S*)-trans-4′-hydroxy-ongokein (**4**)

The spectroscopic properties of compounds **2**–**4**, closely resembled those of (2*S*)-ongokein-4′-one (**1**). ^1H, and ^{13}C NMR data (Table 1) as well as MS fragmentation indicated structures with identical chromanone part, but different cyclohexyl substituents. All NMR resonances of the chromanone protons and carbons were almost identical, and the EIMS of all compounds exhibited the diagnostic fragment ions at *m*/*z* 166 and *m*/*z* 193. The molecular ion of compound **2** was observed at *m*/*z* 352, 46 amu higher than for compound **1**. A fragment ion with *m*/*z* 320 was attributable to the formal loss of one molecule methanol (Δ*m*/*z* 32) and suggested the presence of at least one methoxy group. Consequently, ^1H and ^{13}C NMR resonances of the two methoxy groups of a dimethylketal moiety were observed. The complete structure of **2** was demonstrated by HMQC and HMBC experiments (Fig. 1) as (2*S*)-5-hydroxy-2-(1′-hydroxy-4′,4′-dimethylketal-cyclohexyl)-7-methoxychroman-4-one.

In agreement with **1**, two significant Cotton effects in the CD-curve of **2** (λ 348 nm, [θ]: +966; λ 255 nm, [θ]: −985) elucidated a (2*S*)-configuration (Fukushima et al., 1969; Gaffield, 1970).

Comparable to the conformational situation in the cyclohexane system of **1**, NOE effects confirmed a preferred chair conformation with the bulky chromanone in equatorial orientation. Strong NOE resonances from H-2 (δ 4.20) to the diastereotopic H-2′$_{ax}$ (δ 1.49), and H-6′$_{ax}$ (δ 1.62) show the close spatial proximity, and corroborated the $_{1'}C^{4'}$-chair conformation presented in Fig. 1.

Very similar NMR data and the completely identical EI MS exhibiting key fragments at *m/z* 306 (M^+), *m/z* 193, *m/z* 166, and *m/z* 97 identified compounds **3** and **4** as epimeric 5-hydroxy-2-dihydroxycyclohexyl-7-methoxychroman-4-ones. HMQC, and HMBC experiments confirmed a 1,4-substituted cyclohexyl moiety (Fig. 1). In the ^{1}H NMR of compound **3** the multiplet of H-4′ (δ 3.64) exhibited a broad half-height width ($W_{1/2}$ = 20 Hz), caused by large coupling constants to diaxial oriented protons H-3′$_{ax}$/H-5′$_{ax}$ (Abdullahi et al., 1986; Kuwajima et al., 1998), which demonstrated the axial position of H-4′. On the other hand, the equatorial position of H-4′ in compound **4** was derived from the small half-height width ($W_{1/2}$ = 8 Hz) of its ^{1}H NMR signal, due to smaller coupling constants with all protons located at C-3′, and C-5′. The relative configurations at C-4′ were also confirmed by the typical ^{13}C NMR shifts (Table 1). In agreement with the literature references (Endo et al., 1987; Kalinowski et al., 1984; Günther, 1992) the resonances of the cyclohexane C-4 appeared at δ 70.0 in the ^{13}C NMR of compound **3** and at δ 65.8 in the ^{13}C NMR of compound **4**, respectively. Furthermore the signals of C-2′ and C-6′ appeared at higher field in the spectrum of **4** due to the γ-effect caused by the axial OH-substituent (Günther, 1992).

Subsequently, 1′,4′-*cis* configuration in compound **3** was corroborated by NOEs between H-4′ (δ 3.64) and H-2′$_{ax}$ (δ 1.45) as well as H-6′$_{ax}$ (δ 1.50) (Fig. 1), whereas similar effects were not observed for compound **4**.

The observed Cotton effects for **3** and **4** established the same (2*S*)-configuration as in protoflavanones **1** and **2** (Fukushima et al., 1969; Gaffield, 1970).

Acetylation of **3** and **4** yielded the diacetates **7** and **8** ([M]$^+$ at *m/z* 392 in EI MS), respectively. The *tert*-hydroxy group at C-1′ was not acetylated under the mild conditions used. The observed shifts verified the ^{13}C-assignments in the 1′,4′-cyclohexane ring of **3** and **4**. The C-4′ signals revealed small acetylation-shifts and the α-positions received a remarkable upfield shift by $\Delta\delta \sim 4$ ppm. In comparison to ^{13}C-signals for carbons in β-position to acetylation – C-2′ and C-6′ – remained unchanged, results already observed for similar cyclohexane systems (Abdullahi et al., 1986).

Possibly compounds **1** and **2** might be derived from one another during extraction and separation. However, when treated with MeOH at ambient temperature, no conversion of **1** into the dimethylketal **2** could be observed, even after several weeks, indicating that **2** most probably is a genuine constituent of *O. gore*. On the other hand formation of **1** from **2** only proceeds under strong acidic conditions (see Section 2.4), which were never applied during isolation of the protoflavanones.

2.4. Chemical conversions of protoflavanones *1–4*

Additionally, the structures of compounds **1–4** have been corroborated by chemical conversion of **2–4** into **1**, and of **1** into the corresponding flavonoid (−)-(2*S*)-sakuranetin (**6**) (Mizuno et al., 1987; Arakawa and Nakazaki, 1960) (Fig. 3).

Cleavage of the dimethylketal function of **2** to yield **1** was achieved by treatment with conc. CF_3COOH.

Pyridinium chlorochromate oxidation (Piancatelli et al., 1982) of **3** and **4**, converted both compounds to the 4′-oxo compound **1**. Treatment of **1** with CF_3COOH–H_2SO_4 5:1 afforded a mixture of **5a** and **5b** (Fig. 3), which was dehydrogenated without prior separation by 2,3-dichloro-5,6-dicyano-1,4-benzo-quinone (DDQ) in toluene containing a trace of HCl-gas (Syntex Corp., 1966; Walker and Hiebert, 1967), to yield (−)-(2*S*)-sakuranetin (**6**). No reaction occurred using solely DDQ, and increased HCl concentrations caused complete decomposition of the educts **5a/5b**. ^{1}H NMR, EI MS, and $[\alpha]_D$ of (−)-(2*S*)-sakuranetin (**6**) were in accordance to published spectroscopical data (Mizuno et al., 1987; Arakawa and Nakazaki, 1960).

To get ^{13}C NMR reference data, partial synthesis of (−)-(2*S*)-sakuranetin (**6**) starting from (2*S*)-naringin was accomplished. Applying glycosidase enzyme mixture Rohapect D5L formed the aglycone naringenin. Treatment with highly diluted diazomethane solution (Shinoda and Sato, 1929), achieved selective 7-*O*-methylation and resulted in a quantitative formation of **6**.

2.5. Chemotaxonomic and biosynthetic aspects

So far, flavonoids with partially or completely hydrogenated B-rings were isolated solely from Pteridophyta species (Noro et al., 1969; Fukushima et al., 1969; Hauteville et al., 1980, 1981; Wada et al., 1987; Murakami et al., 1987; Adam, 1999), and were discussed as specific chemotaxonomical markers. Hence, isolation of protoflavonoids from *Ongokea gore* – a spermatophytae plant – is of high interest with respect to chemotaxonomy and biogenesis.

So far, the pathway and its involved enzymes generating flavonoids with hydrogenated B-ring systems remain completely unknown.

During our investigations of different extracts of stem-bark, and roots of *O. gore*, neither any potential biogenetic precursor nor any other flavonoid were detected.

Furthermore, no protoflavanones were found in the photosynthetically active parts (twigs and leaves)

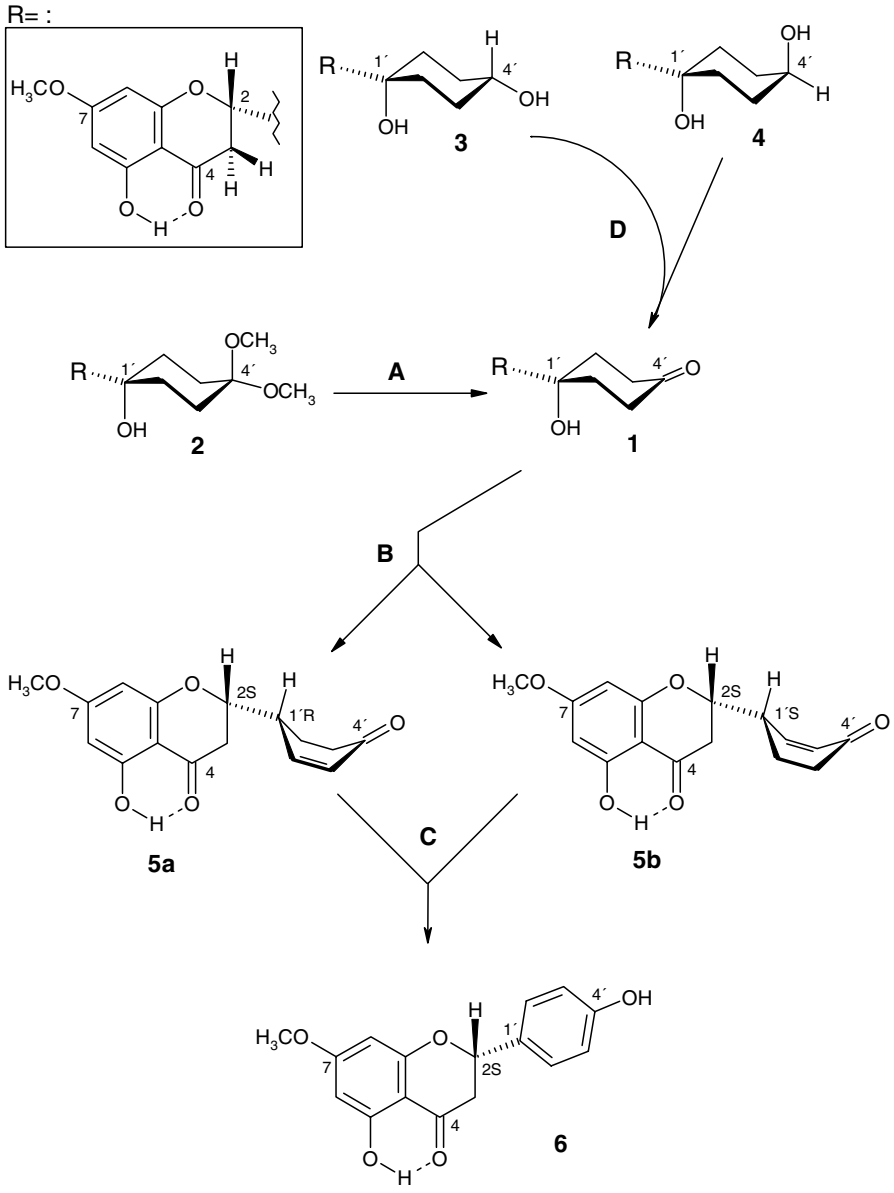

Fig. 3. Chemical conversions of cyclohexanoid flavanones **1**–**4** from *Ongokea gore*: **A**, dimethylketal cleavage of **2** by CF_3COOH addition yielded **1**; **B**, dehydration of *tert*-OH at C-1′ of **1** by CF_3COOH/H_2SO_4 gave diastereomeric pair **5a/5b**; **C**, aromatization of **5a/5b** (2,3-dichloro-5,6-dicyano-1,4-benzoquinone, HCl-gas) to (−)-(*S*)-sakuranetin (**6**); **D**, oxidation of 1′,4′-dihydroxycyclohexyl B-ring moiety of **3** and **4** (pyridinium-chlorochromate) yielded **1**.

indicating that formation of cyclohexanoid based flavonoids is independent of exposure to UV-light.

The available phytochemical data of the Olacaceae plant family are restricted, particularly for flavonoids (Haron and Ping, 1997), therefore, to estimate their chemotaxonomic relevance for the Olacaceae, a systematic investigation of other species of this family for the occurrence of protoflavonoids might be necessary.

2.6. Biological activity

Crude extracts (*n*-hexane, methanol), and solvent partitions (ethylacetate, dichloromethane) of *O. gore* revealed strong cytotoxic activities in the 'brine shrimp'-assay (*A. salina* LEACH) (Meyer et al., 1982), and different antimycotic plate-diffusion-assays. During 'bio-assay' guided fractionation and isolation, we correlated the observed activity to the occurrence of cytotoxic C_{18}-acetylenic fatty acids, already known from the so-called boleko seed oil of *O. gore* (Gunstone and Sealy, 1963; Miller et al., 1977). Investigation of these constituents is in progress.

For the tested protoflavanones **1**, **2** and **4**, the *Artemia*-assay revealed no significant lethal activities in the range beyond *c* 500 ppm (24 h incubation). Podophyllotoxin was used as reference with LD_{50} at a level of *c* 10 ppm.

3. Experimental

3.1. General experimental procedures

Mps uncorr. TLC was performed on precoated plates (Silica gel 60 F_{254}, Merck and C_{18}-nanoplates, Macherey-Nagel) using the following systems: S-1: Silica gel, CH_2Cl_2–MeOH (20:1), S-2: Silica gel, CH_2Cl_2–MeOH (40:1), and S-3: C_{18}-nanoplates, MeOH–H_2O (1:1); detection by UV and anisaldehyde reagent (Stahl and Kaltenbach, 1961). For CC Sephadex® LH20 (Pharmacia) and silica gel 60 0.0040–0.063 mm (Merck) were used. Prep. HPLC on LiChrosorb® RP-18.7 μ (Merck), column dimensions: 2.5 cm i.d. × 25 cm, using MeOH–H_2O mixtures. Unless otherwise stated $[\alpha]_D$ at 21 °C on a Perkin–Elmer polarimeter 241 (cell length: 10 cm), CD on a Jasco spectropolarimeter J-710 at 21 °C (scan range: λ = 200–400 nm, cell length: 0.1 cm). UV–Vis in MeOH (cell length: 1 cm) on a Perkin–Elmer Lambda 5. IR as film on NaCl plates (Jasco FT-IR-410). ^{1}H (360 MHz), and ^{13}C (90.5 MHz) NMR in $CDCl_3$ at 300 K, spectra were recorded on a Bruker AM-360; int. standard TMS for ^{1}H, solvent for ^{13}C NMR. EI MS (70 eV) spectra were measured on a Finnigan TSQ-70 using direct inlet. DCI MS with NH_3, only key ions >15% and m/z > 100 are presented.

3.2. Plant material

Plant material of *O. gore* Engler (Olacaceae) was collected in Ghana (May 1994), near the road between Aburi and Nsawam, approx. 20 km north of Accra, and was identified by the botanist Mr. A. A. Enti (Forestry Enterprises, Ghana). From the healthy, approx. 40 year old tree, the stem-bark (outside: green-grey colour, rough surface like oak, inside: ochre coloured, total thickness 2–3 cm), and the complete roots (ochre colour, ∅ 3–7 cm) were used for the phytochemical investigation. A herbarium specimen is held under No. 94-05 at the Institute of Food Chemistry, Technical University of Braunschweig.

3.3. Extraction and isolation

Lyophilized stem-bark (2260 g) was pulverized in a laboratory mill, exhaustively extracted with petrol to yield 91 g lipophilic extract and with freshly distilled MeOH (82 g of polar extract). The MeOH extract was suspended in 1000 ml MeOH–H_2O (1:2) and then successively re-extracted first with *n*-hexane, and then with CH_2Cl_2 to yield 18 g of CH_2Cl_2 fraction. CC of the CH_2Cl_2 fraction on Sephadex® LH-20 (MeOH) in two portions of 9 g, further CC on SiO_2-60 using CH_2Cl_2–MeOH (20:1) as eluent, and final purification by prep. RP18 HPLC yielded compounds **1**–**4** from the stem-bark extract. MeOH–H_2O (6:4) was used as eluent for the more lipophilic compounds **1** and **2** and MeOH–H_2O (1:1) for the protoflavanones **3** and **4**.

Similar work-up of root material (3000 g) yielded compounds **1**–**4** as minor constituents.

3.4. (2S)-5-Hydroxy-2-(1′-hydroxy-4′-oxocyclohexyl)-7- methoxychroman-4-one (=(2S)-ongokein-4′-one, 1)

White crystals (24 mg from stem-bark, 15 mg from roots). Mp 165–168 °C. TLC: R_f 0.35 (S-1), R_f 0.28 (S-3); anisaldehyde: lilac. Prep. HPLC: (MeOH–H_2O = 6:4, 5 ml/min), R_t = 12 min. $[\alpha]_D$: +36° (*c* 0.13). IR: ν_{max} = 3446 (OH), 2952 (C–H), 1637 (C=O) cm^{-1}. UV: λ_{max} nm (log ε) = 212 (4.18), 226 (4.00), 286 (4.05), 331 (3.35); +NaOH = 242 (4.00), 286 (3.97), 354 (3.56). CD: λ_{max} nm [θ] = 234 (+5556), 270 (−6192), 312 (+4662), 328 (+5682). EI MS: *m/z* (rel. int.): 306 $[M]^+$ (25), 194 (51), 193 (100), 176 (6), 167 (12), 166 (16), 151 (25), 137 (8), 113 (5), 95 (8). ^{1}H NMR: δ = 1.80 (1H, *ddd*, $J_1 = J_2$ = 13.5, J_3 = 5 Hz, H-2′$_{ax}$), 1.88 (1H, *ddd*, $J_1 = J_2$ = 13, J_3 = 5 Hz, H-6′$_{ax}$), 2.05 (1H, *brdd*, J_1 = 13.5, J_2 = 3, Hz, H-2′$_{eq}$), 2.26 (1H, *s*, 1′-O*H*), 2.30–2.42 (3H, *m*, H-3′$_{eq}$, H-5′$_{eq}$, H-6′$_{eq}$), 2.62 (1H, *dd*, J_1 = 17, J_2 = 3 Hz, H-3$_{eq}$), 2.79 (2H, *ddd*, $J_1 = J_2$ = 13, J_3 = 6.5 Hz, H-3′$_{ax}$, H-5′$_{ax}$), 3.01 (1H, *dd*, J_1 = 17, J_2 = 13.5 Hz, H-3$_{ax}$), 3.82 (3H, *s*, 7-OC*H*$_3$), 4.25 (1H, *dd*, J_1 = 13.5, J_2 = 3 Hz, H-2), 6.00 (1H, *d*, *J* = 2 Hz, H-8), 6.07 (1H, *d*, *J* = 2 Hz, H-6), 11.97 (1H, *s*; 5-O*H*). For the ^{13}C NMR spectroscopic data, see Table 1.

3.5. (2S)-5-Hydroxy-2-(1′-hydroxy-4′,4′-dimethoxycyclohexyl)-7-methoxychroman-4-one (=(2S)-4′,4′-dimethoxy-ongokein, 2)

White amorphous powder (12 mg from stem-bark, 19 mg from roots). Mp: 52–55 °C. TLC: R_f 0.32 (S-1), R_f 0.14 (S-3); anisaldehyde: lilac. Prep. HPLC: (MeOH–H_2O = 6:4, 5.0 ml/min), R_t = 22 min. $[\alpha]_D$: +51° (*c* 0.36). IR: ν_{max} = 3450 (OH), 2951 (C–H), 1637 (C=O) cm^{-1}. UV: λ_{max} nm (log ε) = 213 (4.78), 226 (4.61), 286 (4.68), 320 (sh, 3.94); +NaOH = 242 (4.59), 286 (4.60), 355 (4.17). CD: λ_{max} nm [θ] = 240 (+439), 255 (−985), 348 (+966). EI MS: *m/z* (rel. int.) = 352 $[M]^+$ (3), 321 (18), 320 (35), 194 (36), 193 (100), 167 (23), 166 (13), 151 (15), 95 (18). ^{1}H NMR: δ = 1.49 (1H, *ddd*, $J_1 = J_2$ = 13, J_3 = 4.5 Hz, H-2′$_{ax}$), 1.57–1.67 (2H, *m*, H-2′$_{eq}$, H-6′$_{ax}$), 1.80 (2H, *ddd*, $J_1 = J_2$ = 13, J_3 = 4.5 Hz, H-3′$_{ax}$, H-5′$_{ax}$), 1.85–2.00 (3H, *m*, H-3′$_{eq}$, H-5′$_{eq}$, H-6′$_{eq}$), 2.58 (1H, *dd*, J_1 = 17, J_2 = 3 Hz, H-3$_{eq}$), 2.98 (1H, *dd*, J_1 = 17, J_2 = 13.5 Hz, H-3$_{ax}$), 3.16 (3H, *s*; 4′$_{ax}$-OC*H*$_3$), 3.23 (3H, *s*, 4′$_{eq}$-OC*H*$_3$), 3.81 (3H, *s*; 7-OC*H*$_3$), 4.20 (1H, *dd*, J_1 = 13, J_2 = 3 Hz, H-2), 5.98 (1H, *d*, *J* = 2 Hz, H-8), 6.05 (1H, *d*, *J* = 2 Hz, H-6), 12.00 (1H, *s*, 5-O*H*). For the ^{13}C NMR spectroscopic data, see Table 1.

3.6. (2S)-5-Hydroxy-2-(cis-1′,4′-dihydroxycyclohexyl)-7-methoxychroman-4-one (=(2S)-cis-4′-hydroxy-ongokein, **3**)

White crystals (6 mg from stem-bark, 20 mg from roots). Mp: 105–108 °C. TLC: R_f 0.17 (S-1), R_f 0.28 (S-3); anisaldehyde: orange. Prep. HPLC: (MeOH–H_2O = 6:4, 5.0 ml/min), R_t = 24 min. $[\alpha]_D$: +47° (*c* 1.7). IR: ν_{max} = 3391 (OH), 2940 (C–H), 1643 (C=O) cm^{-1}. UV: λ_{max} nm ($\log\varepsilon$) = 213 (4.35), 226 (4.17), 286 (4.23), 331 (sh, 3.50); +NaOH = 242 (4.15), 286 (4.16), 354 (3.73). CD: λ_{max} nm [θ] = 240 (+376), 255 (−760), 347 (+895); EI MS: *m*/*z* (rel. int.) = 308 $[M]^+$ (12), 194 (76), 193 (100), 167 (13), 166 (16), 151 (25), 97 (17). ^{1}H NMR: δ = 1.45 (1H, *ddd*, $J_1 = J_2 = 13.5$, $J_3 = 4$ Hz, H-2′$_{ax}$), 1.50 (1H, *ddd*, $J_1 = J_2 = 13.5$, $J_3 = 4$ Hz, H-6′$_{ax}$), 1.64–1.80 (3H, *m*, H-2′$_{eq}$, H-3′$_{ax}$, H-5′$_{ax}$), 1.87 (2H, *m*, H-3′$_{eq}$, H-5′$_{eq}$), 1.99 (1H, *brs*, 4′-O*H*), 2.10 (1H, *dq*, J_1 = 14, J_2 = 3.5 Hz, H-6′$_{eq}$), 2.60 (1H, *dd*, J_1 = 17, J_2 = 3 Hz, H-3$_{eq}$), 2.97 (1H, *dd*, J_1 = 17, J_2 = 13.5 Hz, H-3$_{ax}$), 3.48 (1H, *s*; 1′-O*H*), 3.64 (1H, *brm*, H-4′$_{ax}$), 3.80 (3H, *s*, 7-OC*H*$_3$), 4.15 (1H, *dd*, J_1 = 13.5, J_2 = 3 Hz, H-2), 5.98 (1H, *d*, *J* = 2 Hz, H-8), 6.04 (1H, *d*, *J* = 2 Hz, H-6), 11.99 (1H, *s*, 5-O*H*). For the ^{13}C NMR spectroscopic data, see Table 1.

3.7. (2S)-5-Hydroxy-2-(trans-1′,4′-dihydroxycyclohexyl)-7-methoxychroman-4-one (=(2S)-trans-4′-hydroxy-ongokein, **4**)

White amorphous powder (48 mg from stem-bark, 170 mg from roots). Mp: 68–71 °C. TLC: R_f 0.21 (S-1), R_f 0.28 (S-3); anisaldehyde: orange. Prep. HPLC: (MeOH–H_2O = 1:1, 5.50 ml/min), R_t = 40 min. $[\alpha]_D$: +68° (*c* 0.69). IR: ν_{max} = 3420 (OH), 2934 (C–H), 1644 (C=O) cm^{-1}. UV: λ_{max} nm ($\log\varepsilon$) = 213 (4.76), 226 (4.60), 286 (4.66), 326 (sh, 3.92); +NaOH = 242 (4.55), 286 (4.59), 354 (4.13). CD: λ_{max} nm [θ] = 242 (+70), 250 (−275), 358 (+312). EI MS: *m*/*z* (rel. int.) = 308 $[M]^+$ (11), 194 (69), 193 (100), 176 (10), 167 (15), 166 (19), 151 (23), 137 (5), 97 (12), 69 (7). ^{1}H NMR: δ = 1.48 (1H, *m*, H-2′$_{ax}$), 1.56–2.01 (7H, *m*, H-2$_{eq}$, H-3′$_{eq}$, H-3′$_{ax}$, H-5′$_{eq}$, H-5′$_{ax}$, H-6′$_{eq}$, H-6′$_{ax}$), 2.07 (1H, *brs*, 1-O*H*), 2.60 (1H, *dd*, J_1 = 17, J_2 = 2.5 Hz, H-3$_{eq}$), 2.98 (1H, *dd*, J_1 = 17, J_2 = 13 Hz, H-3$_{ax}$), 3.47 (1H, *s*, 4′-O*H*), 3.80 (3H, *s*, 7-OC*H*$_3$), 4.07 (1H, *m*, $W_{1/2}$ = 8 Hz, H-4′$_{eq}$), 4.25 (1H, *dd*, J_1 = 13, J_2 = 3 Hz, H-2), 5.99 (1H, *d*, *J* = 2 Hz, H-8), 6.04 (1H, *d*, *J* = 2 Hz, H-6), 12.00 (1H, *s*, 5-O*H*). For the ^{13}C NMR spectroscopic data, see Table 1.

3.8. Chemical conversion of **2** into **1**

Treatment of **2** with CF_3COOH (abs) for 2 h at room temperature, neutralization with sat. aqueous $NaHCO_3$-solution, extraction with CH_2Cl_2 and evaporation of the combined extracts yielded **1**. The spectroscopic properties completely agreed with those of (2*S*)-ongokein-4′-one of plant origin.

3.9. Oxidation of **3** and **4** with pyridinium chlorochromate (PCC) (Piancatelli et al., 1982) to **1**

Compound **3** (or **4**) (33 mg) was treated with PCC in CH_2Cl_2 (room temperature, 3 h) to yield **1** (5 mg) after purification by CC (∅ 1 cm, length 20 cm, SiO_2 (5 g); CH_2Cl_2–MeOH, 20:1). The properties of the reaction products were identical with **1**.

3.10. Chemical conversion of **1** into a mixture of **5a** and **5b**

Compound **1** (11 mg) was dissolved in 2 ml CF_3COOH (abs.) at 0 °C and 10 drops H_2SO_4 (abs.) were added slowly. After standing for 5 h at room temperature, the products were extracted with CH_2Cl_2 and purified by CC (SiO_2, CH_2Cl_2–MeOH = 80:1) to yield 5 mg of diastereomers **5a** and **5b** as an amorphous powder (5 mg). TLC: R_f 0.52 (S-2); anisaldehyde: green-black. UV: λ_{max} nm ($\log\varepsilon$) = 226 (4.28), 287 (4.14), 327 (3.41); +NaOH = 289 (4.02), 339 (3.72), 456 (3.58), 576 (3.65). CD: λ_{max} nm [θ] = 245 (+161), 262 (−388), 316 (+647), 328 (+970). EI MS: *m*/*z* (rel. int.) = 288 $[M]^+$ (15), 194 (12), 193 (100), 165 (13),137 (12), 95 (5). ^{1}H NMR: δ = 1.85–2.05 (1H, *ddd*, $J_1 = J_2 = 13.5$, $J_3 = 5$ Hz, H-6′$_{ax}$), 2.20 (1H, *m*, H-5′$_{eq}$), 2.45 (1H, *m*, H-5′$_{ax}$), 2.65 (1H, *m*, H-5′$_{ax}$), 2.62 (1H, *dd*, J_1 = 17, J_2 = 3 Hz, H-3$_{eq}$), 2.65 (1H, *dd*, J_1 = 17, J_2 = 3 Hz, H-3$_{eq}$), 2.76–2.95 (3H, *m*, H-1′, H-3$_{ax}$, H-3$_{ax}$), 3.82 (3H, *s*, 7-OC*H*$_3$), 3.83 (3H, *s*, 7-OC*H*$_3$), 4.36 (1H, *ddd*, J_1 = 13, J_2 = 6.5, J_3 = 3 Hz, H-2), 4.51 (1H, *ddd*, J_1 = 13, J_2 = 5, J_3 = 3 Hz, H-2), 6.00 (1H, *d*, *J* = 2.5 Hz, H-8), 6.02 (1H, *d*, *J* = 2.5 Hz, H-8), 6.06 (1H, *d*, *J* = 2.5 Hz, H-6), 6.07 (1H, *d*, *J* = 2.5 Hz, H-6), 6.13–6.20 (2H, H-3, H-3′, 6.96 (1H, *dq*, J_1 = 10, J_2 = 1 Hz, H-2′, 7.13 (1H, *dq*, J_1 = 10, J_2 = 1Hz, H-2′, 11.93 (1H, *s*, 5-O*H*), 11.94 (1H, *s*, 5-O*H*). For the ^{13}C NMR spectroscopic data, see Table 1.

3.11. Chemical conversion of the mixture of **5a** and **5b** to (−)-(S)-Sakuranetin (**6**)

To **5a/5b** (5 mg) dissolved in 2 ml toluene, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (20 mg) was added. A trace of HCl-gas (Syntex Corp., 1966; Walker and Hiebert, 1967) was bubbled through the reaction mixture (4 s) and the mixture was kept at room temperature for 12 h. Purification by CC (CH_2Cl_2–MeOH = 40:1) yielded a product (1 mg) identical with synthesized (−)-(*S*)-sakuranetin (**6**).

3.12. Partial synthesis of (−)-(S)-sakuranetin (**6**)

Enzymatic hydrolysis of 30 mg (−)-(*S*)-naringin (SUNKIST Growers Inc., California, USA) by glucosidase

enzyme Rohapect® D5L (RÖHM, Germany) in citric–phosphorous acid McIllvine buffer (pH 5.5, 37 °C, 24 h) yielded 12 mg (−)-(*S*)-naringenin, 5 mg of which were selectively methylated by diluted diazomethane solution (MeOH–Et_2O = 1:4) at room temperature for 6 h) to give (−)-(*S*)-sakuranetin (**6**) in quantitative yield (Shinoda and Sato, 1929) as ochre crystals (1 mg). Mp. 122–124 °C. TLC: R_f 0.42 (S-1); anisaldehyde: orange. $[\alpha]_D$: −9° (*c* 0.28). UV: λ_{max} nm (log ε) = 226 (4.19), 286 (4.02), 337 (3.42), 398 (3.07); +NaOH = 246 (4.05), 287 (3.90), 399 (3.80). IR: ν_{max} = 3373 (OH), 1642 (C=O) cm^{-1}. EIMS: *m/z* (rel. int.) = 287 $[M + 1]^+$ (17), 286 $[M]^+$ (100), 285 (61), 193 (41), 180 (53), 167 (99), 138 (35), 120 (44), 95 (29), 91 (23). ^1H NMR: δ = 2.79 (1H, *dd*, J_1 = 13.5, J_2 = 3.5 Hz, H-3_{eq}), 3.09 (1H, *dd*, J_1 = 17, J_2 = 13.5 Hz, H-3_{ax}), 3.80 (3H, *s*, 7-OCH_3), 5.02 (1H, *brs*, 4′-O*H*), 5.35 (1H, *dd*, J_1 = 13.5, J_2 = 3 Hz, H-2), 6.05 (1H, *d*, *J* = 2 Hz, H-8), 6.07 (1H, *d*, *J* = 2 Hz, H-6), 6.89 (2H, AA′-*BB*′, H-3′, H-5′), 7.35 (2H, *AA*′-BB′, H-2′, H-6′) 12.03 (1H, *s*, 5-O*H*). For the ^{13}C NMR spectroscopic data, see Table 1 Authenticity of (−)-(*S*)-naringin, and (−)-(*S*)-naringenin were verified by EI MS, ^1H, and ^{13}C NMR measurements.

3.13. Diacetate of 3

Compound **3** (mg) was treated with pyridine-Ac_2O = 1:1 at room temperature for 18 h. Evaporation yielded the **7** as an amorphous powder. TLC: R_f 0.23 (S-2); anisaldehyde: orange. EI MS: *m/z* (rel. int.) = 392 $[M]^+$, 350 (9), 195 (10), 194 (96), 193 (100), 176 (7), 167 (12), 166 (17), 151 (22), 137 (5), 97 (14). ^1H NMR: δ = 1.43–1.60 (2H, *m*, H-2′$_{ax}$, H-6′$_{ax}$), 1.70–1.92 (6H, *m*, H-2′$_{eq}$, H-6′$_{eq}$, H-3′$_{ax}$, H-5′$_{ax}$, H-3′$_{eq}$, H-5′$_{eq}$), 2.00 (3H, *s*, -CO-CH_3), 2.10 (1H, *s*, 1′-O*H*), 2.37 (3H, *s*, -CO-CH_3), 2.52 (1H, *dd*, J_1 = 17, J_2 = 3 Hz, H-3_{eq}), 2.87 (1H, *dd*, J_1 = 17, J_2 = 13.5 Hz, H-3_{ax}), 3.83 (3H, *s*, 7 -OCH_3), 4.22 (1H, *dd*, J_1 = 13, J_2 = 3 Hz, H-2), 4.72 (1H, *m*, H-4′$_{ax}$), 6.26 (1H, *d*, *J* = 2 Hz, H-8), 6.37 (1H, *d*, *J* = 2 Hz, H-6). For the ^{13}C NMR spectroscopic data, see Table 1.

3.14. Diacetate of 4

Acetylation of compound **4** as described for **3** yielded **8** as an amorphous powser. TLC: R_f 0.26 (S-2); anisaldehyde: orange. EI MS: *m/z* = 392 $[M]^+$ (0.1), 350 (3), 236 (31), 195 (24), 194 (100), 193 (67), 176 (7), 167 (10), 166 (12), 151 (17), 137 (5), 97 (9); DCI MS: *m/z* = 393 $[M + 1^+]$ (100), 351 (15). ^1H NMR: δ = 1.43–1.60 (2H, *m*, H-2′$_{ax}$, H-6′$_{ax}$), 1.65–1.92 (6H, *m*, H-2′$_{eq}$, H-3′$_{eq}$, H-3′$_{ax}$, H-5′$_{eq}$, H-5′$_{ax}$, H-6′$_{eq}$), 1.98 (3H, *s*, –CO–CH_3), 2.30 (3H, *s*, -CO-CH_3), 2.47 (1H, *dd*, J_1 = 17, J_2 = 2.5 Hz, H-3_{eq}), 2.83 (1H, *dd*, J_1 = 17, J_2 = 13 Hz, H-3_{ax}), 3.47 (1H, *s*, 1′-O*H*), 3.80 (3H, *s*, 7-OCH_3), 4.23 (1H, *dd*, J_1 = 15, J_2 = 2.5 Hz, H-2), 4.98 (1H, m_s, H-4′$_{eq}$), 6.30 (1H, *d*, *J* = 2 Hz, H-8), 6.25 (1H, *d*, *J* = 2 Hz, H-6). For the ^{13}C NMR spectroscopic data, see Table 1.

Acknowledgements

The 'Deutsche Forschungsgemeinschaft', and 'Fonds der Chemischen Industrie' are thanked for financial support. Also, many thanks are due to Mrs. B. Barnowski (Institute of Pharmacy and Food Chemistry, University Erlangen-Nürnberg) for performing 1D-, and 2D NMR experiments. Particularly, we are grateful to Mr. Albert A. Enti (Forestry Enterprises, Legon, Ghana) for his assistance in collection and identification of the plant material.

References

Abdullahi, H., Nyandat, E., Galeffi, C., Messana, I., Nicoletti, M., Bettolo, G.B.M., 1986. Cyclohexanols of *Halleria lucida*. Phytochemistry 25, 2821–2823.

Adam, K.-P., 1999. Phenolic constituents of the fern *Phegopteris connectilis*. Phytochemistry 52, 929–934.

Arakawa, S., Nakazaki, M., 1960. Die absolute Konfiguration der optisch aktiven Flavanone. Chem. Ber. 636, 111–117.

Audier, H., 1966. Étude des composés flavoniques par spectrométmetrie de masse. Bull. Soc. Chim. France 9, 2892–2899.

Endo, K., Seya, K., Hikino, H., 1987. Stereostructure of rengyol and isorengyol, phenylethanoids of *Forsythia suspensa*. Tetrahedron 43, 2681–2688.

Fukushima, S., Noro, T., Akahori, Y., Saiki, Y., Ueno, A., 1969. Studies on the constituents of *Leptorumohra miqueliana*H. Ito. Part III. The conformation of protofarrerol. Yakugaku Zasshi 89, 1272–1275.

Gaffield, W., 1970. Circular dichroism, optical rotatory dispersion and absolute configuration of 3-hydroxyflavanones and their glycosides. Tetrahedron 26, 4093–4108.

Günther, H., 1992. NMR-Spektroskopie. Georg Thieme Verlag, Stuttgart, New York, p. 78 and pp. 448–449.

Gunstone, F.D., Sealy, A.J., 1963. Fatty acids. Part XII. The acetylenic acids of isano (boleko) oil. J. Chem. Soc., Perkin Trans. 1, 5772–5778.

Haron, W.H., Ping, S.T., 1997. Distribution and taxonomic significance of flavonoids in the Olacaceae and Icacinaceae. Biochem. Syst. Ecol. 25, 263–265.

Hauteville, M., Chopin, J., Geiger, H., Schüler, L., 1980. Protogenkwanin 4′-glucoside, a new type of natural flavonoid with a non aromatic B-ring. Tetrahedron Lett. 21, 1227–1230.

Hauteville, M., Chopin, J., Geiger, H., Schüler, L., 1981. Protogenkwanin, a new flavonoid from *Equisetum arvense* L. Tetrahedron 37, 377–381.

Hegnauer, R., 1990Chemotaxonomie Der Pflanzen, 9. Birkhäuser Verlag, Basel, Boston, Berlin, p. 156.

Irvine, F.R., 1961. Woody Plants of Ghana. Oxford University Press, London, p. 467.

Kalinowski, H.-O., Berger, S., Braun, S., 1984. ^{13}C-NMR-Spektroskopie. Georg Thieme Verlag, Stuttgart, New York, pp. 236–241.

Kuwajima, H., Takai, Y., Takaishi, K., Inoue, K., 1998. Synthesis of ^{13}C-labeled possible intermediates in the biosynthesis of phenylethanoid derivates, cornosides and rengyosides. Chem. Pharm. Bull. 46, 581–586.

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., McLaughlin, J.L., 1982. Brine shrimp: a convenient bioassay for active plant constituents. Planta Med. 45, 31–34.

Miller, R.W., Weisleder, D., Kleiman, R., Plattner, R.D., Smith Jr., C.R., 1977. Oxygenated fatty acids of isano oil. Phytochemistry 16, 947–951.

Mizuno, M., Kojima, H., Tanaka, T., Iinuma, M., Kimura, R., Zhi-Da, M., Murata, H., 1987. Phenolic constituents from seeds of *Coptis japonica* var. dissecta. Phytochemistry 26, 2071–2074.

Murakami, T., Kido, T., Hori, K., Satake, T., Saiki, Y., Chen, C.-M., 1987. Chemical and chemotaxonomic studies of filices. LXVII. The distribution of a flavanone with a modified B-ring, protofarrerol and its derivates. Yakugaku Zasshi 107, 416–419.

Noro, T., Fukushima, S., Saiki, Y., Ueno, A., Akahori, Y., 1969. Studies on the constituents of *Leptorumohra miqueliana* H. Ito. Part II. The structure of protofarrerol. Yakugaku Zasshi 89, 851–856.

Piancatelli, A., Scettri, A., D'Auria, M., 1982. Pyridinium chlorochromate: A versatile oxidant in organic synthesis. Synthesis, 245–258.

Shinoda, J., Sato, S., 1929. Neue Synthesen von Polyoxychalkonen, -hydroxychalkonen, und -flavanonen. II. Synthese des Naringenins und Sakuranetins. Chemisches Zentralblatt 100, 244.

Stahl, E., Kaltenbach, U., 1961. Dünnschicht-Chromatographie, VI. Mitteilung. Spurenanalyse von Zuckergemischen auf Kieselgur G-Schichten. J. Chromatogr. 5, 351–355.

Syntex Corp., Neth. Appl. 299,968, 1966. Synthesis 6-dehydro-19-norprogesterone. Chemical Abstracts 64, 3644b.

Wada, H., Fujita, H., Murakami, T., Saiki, Y., Chen, C.-M., 1987. Chemical and chemotaxonimical studies of ferns LXXXIII. New flavonoids with modified B-ring from the genus *Pseudophegopteris* (Thelypteridaceae). Chem. Pharm. Bull. 35, 4757–4762.

Waibel, R., Benirschke, G., Benirschke, M., Achenbach, H., 2003. Sesquineolignans and other constituents from the seeds of *Joannesia princeps*. Phytochemistry 62, 805–811.

Walker, D., Hiebert, J.D., 1967. 2,3-Dichloro-5,6-dicyanobenzoquinone and its reactions. Chem. Rev. 67, 153–195.