Bioactive phenolic constituents of some Ochnaceae of Cameroon

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ABSTRACT 25 Bioactive polyflavonoids from *Lophira alata*, *L. lanceolata* and *Ochna calodendron* were discussed. Structural elucidation was performed by spectral methods, notably 2D NMR: COSY, HMBC and NOESY.

ABSTRAK 25 Poliflavonoid yang bioaktif dari *Lophira alata*, *L. lanceolata* dan *Ochna calodendron* dibincangkan. Penentuan struktur dijalankan dengan kaedah spektroskopi, khususnya 2D NMR: COSY, HMBC, dan NOESY.

(Ochnaceae, polyflavonoids, *Lophira alata*, *Lophira lanceolata*, *Ochna calodendron*)

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INTRODUCTION

Ochnaceae is a large pantropical family of trees, shrubs and herbs enclosing about forty genera and six hundred species. In Cameroon this family is represented by several trees, *Lophira alata*, termed the african oak and *Ochna calodendron* both represent tall trees of the african rain forest, and *L. lanceolata* a smaller tree which is widely distributed in the woody savanna of tropical Africa. Due to its resistance to fungal degradation *L. alata* was used for railway sleepers. The barks of these trees are used in African folk medicine for treatment of tooth-ache in Cameroon, for liver infection in Togo and for dysentery and cough in Nigeria. We observed that the extracts and some of the purified constituents exhibit antifungal and antibacterial activities, especially against *S. aureus* and some microscopic fungi \([1-14]\). Furthermore an action as inhibitors of the Epstein-Barr virus-activation induced by tumor promoters like telocidin B-4 was pointed out by Koshimizu and coworkers \([15-18]\).

Preliminary observations suggested the presence of flavonoids in the barks of these species thus prompt us to develop a research program for antimicrobial products from the Cameroonian Ochnaceae. Some plants of this family are known to biosynthesize biflavones such as ochnatlavone, a biflavonyle ether \([19-20]\) and isocharmadejasmin, a dimeric flavanone \([21]\), and also biflavonones such as amentoflavone derivatives. *Brackenridgea zanguebarica*, a small tree occurring in the Northern Transvaal and Zambia, is used in folk medicine for the treatment of wounds. It contains the dimeric dihydrochalcone, brackenin \([22, 23]\).

Our group examined the flavonoid content of the bark of the two *Lophira* species and of *Ochna calodendron*. The barks, which presented yellow and orange brown layers, were dried, pulverized, extracted with ethyl acetate and the extracts fractionated by repetitive chromatography. The structures of the purified compounds were determined by spectral methods including mass spectrometry and NMR notably the 2D methods \(^{1}H-^{1}H\text{ COSY, }^{1}H-^{13}C\text{ COSY, HMBC and NOESY}.\)
RESULTS AND DISCUSSION

More than 25 flavonoids Ochnaceae were isolated from the trunk bark extracts of the above Ochnaceae, among which a special group of oligomers (bi-, tetra- and hexamers) formed from chalcones as building blocks [1-14] (Table 1). All of them were derivatives of the chalcone isoliquiritigenin and could be ranked into three groups, bi-, tetra- and hexaflavonoids, according to the number of chalcone units involved, two, four or six, respectively. The flavonoid group could be further classified into several subgroups, depending on the mode of linkage of the flavonol units: the α-carbon of one isoliquiritigenin unit can be linked to the α, to the 3 or to the 3' position of the second unit, and thus were defined the (α, 3), the (α, 3') and the (α,α) types. The variety of the isolated flavonoids arise from the diversity of further reduction, oxidation and cyclisation pathways.

A typical biflavonoid structure of the (α, 3) type is the yellow crystalline lophiron C, the dihydrobenzofuran ring arises from the trans-addition of the 4-hydroxyl group of the first chalcone unit to the α, β double bond of the second unit [3] (Figure 1). The trans relative disposition of the dihydrobenzofuran substituents was determined by NOE measurements. The isomeric compound lophiron B is formed by trans addition of the 2'-hydroxyl group of the second chalcone unit on its own α, β double bond. Lophirones K, D and E, all with yellow to orange colors, are formally derived from lophiron C by oxidation of the carbon α2 or by cleavage of the phenacyl group at position α2 on either side of the carbonyl group, respectively [4,11]. Bongosin is a flavanone-chalcone dimer in which the carbonyl group of the flavanone is reduced [6]. This compound is suggested to be biosynthetically related to lophiron B.

Table 1 Polyflavonoids isolated from of the stem bark of Lophira alata (LA), L. lanceolata (LL) and Ochna calodendron (OC).

<table>
<thead>
<tr>
<th>Compound</th>
<th>LA</th>
<th>LL</th>
<th>OC</th>
<th>Molecular Formula</th>
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<tr>
<td>Lophiron A +</td>
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<td>+</td>
<td></td>
<td>C_{36}H_{27}O_{8}</td>
</tr>
<tr>
<td>Lophiron B +</td>
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<tr>
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<tr>
<td>Bongosin</td>
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<tr>
<td>Calodenin A</td>
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Calodenins A and B, which were isolated only from *O. calodendron*, are representative biflavonoids of the (α,3')-type [11]. The structure determination of calodenin A, and NMR assignment of its phenolic groups, arose from the analysis of the HMB spectrum which showed correlations between the hydroxyl protons and the carbon atoms linked to the hydroxyl as well as the vicinal carbons (Figure 1). Calodenin B, an α,β-dehydroderivative, was previously isolated from *B. zanguebarica*, together with its dihydrobenzofuran derivative and brackenin. This latter results from the linkage of the α-carbon of the dihydrochalcone precursor [22, 23].

Chamaejasmin, a biflavonone previously described in *Stellera chamaejasme* and which also results from the linkage at the α-carbon of two chalcone units, was isolated from *O. calodendron*.

Lophitone A, a colorless compound which is the main constituent of the three cameroonian species, is a rearranged biflavonoid which may be formed from such a precursor such as chamaejasmin, according to the pathway described in figure 2 [1]. This scheme was also proposed for the formation of chamaechromone, a rearranged flavonoid from *Stellera chamaejasme* (Thymelaeaceae) [24]. However, to explain the formation of these compounds, a radical process should not be excluded. Calodenone isolated from *O. calodendron* is an O-methyl derivative of lophitone A [9].

An important subgroup of biflavonoids of the (α, α)-type encloses lophirones F and G on one hand, and lophitone H, mbamiichalcone and isombamichalcone on the other hand [2, 5, 7]. All these colorless compounds are characterized by a tetrahydrofuran moiety, each being substituted by a phenyl, a benzyl or a benzyl group. Lophirones F and G have the same plane structure and differ between themselves by the stereochemistry of carbon β1. Their relative stereochemistry was determined from NOE difference measurements, as shown in figure 3 [7]. The NMR spectrum of lophitone G had only signals for half of the protons of the molecular formula, indicating a symmetry in the molecule. The measured NOE value (6%) between H-α1 and H-α2 is the sum of the NOEs between H-α1 and both H-β1 and H-β2, because signals for H-α1 and H-α2, as well as for H-β1 and H-β2, are superimposed in the NMR spectrum. As lophitone G has a rotatory power, the symmetry is axial and not planar, thus leading to the proposed relative stereochemistry, with trans-trans-trans substitution on the tetrahydrofuran ring; a trans-cis-trans derivative would have a planar symmetry and would be devoid of optical activity.

![Figure 1. Biflavonoids of the Ochnaceae: (α-3) and (α-3') type of interflavonoyl linkage](image-url)

Lophitone C: R₁ = H  
Lophitone K: R₁ = OH

Lophitone B

Lophitone D: R₁ = CHO  
Lophitone E: R₁ = H

Calodenin A
Figure 2. Formation of lophirone A and related compounds from a bilafavone precursor.

Figure 3. NOE difference data for lophirone F and relative stereochemistry of lophinines F and G.

Mbamichalcone and isombamichalcone are two isomeric tetrahydrofuran derivatives which differ from lophinones F and G by the relative orientation of the chalcone units: they are antiparallel instead of parallel [2,5]. Moreover, they have a methylene group instead of a carbonyl group at position β1. Mbamichalcone and isombamichalcone have been shown, from NOE difference measurements, to be epimers at carbon C1 (Figure 4). They should be related to cordigone, a chalcone from B. zanguebarica which has a carbonyl group at β1 [25].
Lophirone H is structurally derived from isombamchalcone by a cyclisation involving carbon atom at 2' on A1-ring and carbon atom B1, which are linked by an oxygen bridge [7]. Lophirone H is also related to cordigol [25], a biflavonoid of *B. zanguebarica* which has the same relative stereochemistry for the fused heterocyclic ring system.

The basic structure of all these complex biflavonoid structures is isoliquiritigenin (Figure 5). They consist in dimers arising by three distinct routes. In the first, the α-position of one chalcone unit becomes bonded to the α-carbon of the second unit. Further cyclization, involving either a parallel or an antiparallel disposition of the chalcone units, formed a shared tetrahydrofuran ring, such as for lophirone F and mbamchalcone, respectively. Variation in stereochemistry of the substituents on the tetrahydrofuran ring and secondary molecular modifications account for the formation of other related dimers. The second mode of dimerization involves a bond formation between the α-carbon of one chalcone unit and the 3-position (B ring) of the second unit, probably by phenolic coupling, leading to such products as lophirones B and C and bongosin. Lophirones D and E are thought to be the degradation products of lophirone C through elimination of a phenyl and a benzoyl group, respectively. In the third observed way of dimerization, the α-carbon of one chalcone unit is linked to the 3'-position (A ring) of the second unit, leading to such compounds as calodenins A and B.

A series of tetrafлавonoids, lophirachalcone and lophiroflavans A-C which are clearly arising through condensation of two biflavonoid units, one being of the (α,α)-type, and the second of the (α,3')-type (Figure 6).

Structure analysis showed that lophiroflavans B and C are formed from lophirone H, the C-2 position of which is linked to the C-5' aromatic carbon on the A1-ring of mbamchalcone and isombamchalcone, respectively [10]. Further coupling of the hydroxyl at 4' on this A1 ring with the β2 carbon of lophirone H formed a dehydropyran ring, leading to lophiroflavan A [8].
Figure 5. Biflavonoids of the Ochnaceae. Different modes of interflavonoyl linkage.
Lophirachalcone is synthesised by similar coupling between the C-2 carbon of lophirone C and the C-5' aromatic carbon on the A_{1}-ring of isombamichalcone [6]. Two of its isomers, isolphirachalcone and alatachalcone, were isolated by Koshimizu et al: the first one is the epimer at C3 of lophirachalcone, involving mbamichalcone instead of isombamichalcone as building block, and the second one involves lophirone B and mbamichalcone as biflavonoid subunits [15, 18].

From the more polar fractions of the *L. alata* bark extract, was isolated the hexaflavonoid lophira-hexachalcone (Figure 7). Structural analysis showed it was composed of three biflavonoid units, one of the (αα,)-type (isombamichalcone), and two of the (α,3)-type (lophirone C).

Figure 6. Selected structures of tetraflavonoids of the *Lophira*.

Figure 7. Structure proposed for the hexaflavonoid lophira-hexachalcone.
REFERENCES


