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Curare Alkaloids: Constituents of a Matis Dart Poison

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ABSTRACT: A phytochemical study of dart and arrow poison from the Matis tribe led to the identification of D-(−)-quinic acid, L-malic acid, ethyldimethylamine, magnoflorine, and five new bisbenzyltetrahydroisoquinoline alkaloids (BBIQAs), I−5. D-Tubocurarine could not be identified among these products. BBIQA (3) contains a unique linkage at C-8 and C-11. All structures were characterized by a combination of NMR and HRESIMS data. The effects of Matis poison and individual BBIQAs (1−3) on rat muscle nAChR expressed in Xenopus oocytes have been investigated using the two-electrode voltage clamp technique.

During the course of their evolution, many plant species have survived through their ability to synthesize and accumulate toxic compounds that protect them against microorganisms, insects, herbivores, or even other plants. Some of these toxins have found a role in the subsistence activities of traditional societies, providing a source of arrow and fish poisons. In nearly all parts of the world, hunting poisons prepared from extracts of poisonous plants have been used as auxiliary weapons in the procurement of food and clothes.1,2 Indeed, people worldwide made their hunting trips more effective by applying poisonous materials to arrows, darts, spears, or javelins.

Arrow poisons from South America are a rich natural source of alkaloids from several genera of the Menispermaceae [Abuta, Chondrodendron (especially C. tomentosum), Curarea, Sciadotea, and Telitoxicum]3,4 as well as the Longanaceae (Strychnos).5 Frogs of the family Dendrobatidae are also known as a source of arrow poison, e.g., Phyllobates bicolor, P. aurotaenia, and P. terribilis.6,7 Chondrodendron tomentosum Ruiz. & Pav. is the main species used by South American Indians as an arrow poison in various blends of curare. Curare is, by definition, a black resin obtained from certain tropical South American trees, containing bisbenzyltetrahydroisoquinoline alkaloids,8 especially d-tubocurarine, whose action on the skeletal muscle end plate acetylcholine receptor (AchR) resulting in muscular paralysis is well known.9

Many scientists have been fascinated by curare,10 and the chemical and pharmacological studies of the active principles led to the discovery of pharmacologically active compounds still used around the globe.11 Introduction of curare in clinical medicine in the first half of the last century represented a major breakthrough in the areas of anesthesia and surgery.12−14 The use of curare permitted the development of the concept of the so-called “balanced anesthesia”,15 in which the three components of general anesthesia, i.e., unconsciousness, analgesia, and muscle relaxation, are provided by separate drugs, e.g., thiopental, nitrous oxide, opioids, and curare.

The Matis tribe, also known as the Jaguar People, is a hunting tribe originating from western Brazil. They are specialist hunters known to use poison from frogs (Kampo) and many toxic plants for this purpose.16,17 The tribe employs mainly blowpipes, the construction of which is rather complex. For the darts thin palm stems are tipped with poison extracted from a yet unknown vine. The vine is scraped with a stick embedded with monkey teeth, and the mixture is then boiled and concentrated for approximately 2 weeks. In the Matis tribe, some of these poisons have also been employed as ingredients in or are sources of traditional remedies.18 Arrow and ordeal poisons are still considered as conventional natural sources for future drug discovery.19 Moreover, they have been and still are an important source of pharmacological tools, as well as a valuable source of original chemical structures for the development of new drugs. Thus, a phytochemical investigation of the Matis poison was performed to identify its bioactive compounds. This led to the isolation and characterization of D-(−)-quinic acid, L-malic acid, ethyldimethylamine,
magnoflorine, and bisbenzyltetrahydroisoquinoline alkaloids (1–5).

Commercially available d-tubocurarine chloride was used to compare its spectroscopic properties with the isolated bisbenzyltetrahydroisoquinoline alkaloid derivatives. However, d-tubocurarine itself could not be identified among these products. The effects of crude Matis poison and individual bisbenzyltetrahydroisoquinoline alkaloids (BBIQAs) (1–3) on rat muscle nAChR expressed in Xenopus oocytes have been investigated using the two-electrode voltage clamp technique.

**RESULTS AND DISCUSSION**

Spot tests were used for the qualitative determination of secondary metabolites present in the Matis poison.25,26 We identified steroids and triterpenoids (dark green) by the Liebermann–Burchard test, flavonoids (red) by the Shinoda test, and alkaloids by using Dragendorff’s, Mayer’s, and Wagner’s reagents and the picric acid test. No saponins were detected. However, BBIQAs can be confused with saponins in the foam test, but no saponins were detected by 1H NMR spectroscopy.

The dried poison (5.29 g) was purified to yield three fractions. Fraction A consisted of small pieces of bark, stem, and leaves. Fraction B yielded 12.3 mg of d-(−)-quinic acid and 3.4 mg of L-malic acid. The structures of d-(−)-quinic acid and L-malic acid were elucidated by means of spectroscopic data comparison with literature values.21

Fraction C yielded ethyldimethylamine, magnoflorine, and compounds 1–5. Ethyldimethylamine was identified by means of data comparison with literature results.

![Figure 1. Structure of magnoflorine.](image)

Magnoflorine was isolated as a pale yellow resin.13C NMR and HRESIMS data indicated a molecular formula of C20H24NO4 based upon the protonated molecular ion at m/z 342.1706 (calculated for C20H24NO4+ : 342.1722). The 1H and 13C NMR data are in accordance with literature data for magnoflorine.25 From 1H, 13C, and edited HSQC spectra characteristic absorptions for three methylene groups (δH 3.22/2.80, δC 23.33; 3.61/3.46, 61.12 and 3.08/2.62, 30.40), two methoxy groups (δH 3.80, δC 54.68; 3.82, 54.91), two N-methyl groups (δH 3.30, δC 52.53 and 2.90, 42.11), and three aromatic protons (δH 6.61 s, δC 108.25; 6.62 d, 116.05; 6.74 d, 109.24; J = 8.0 Hz) were evident. The HMBC data showed the expected correlations corresponding to the condensed ring system.

Magnoflorine has been described as a ‘weak’ neuromuscular blocking agent and a not-so-weak ganglionic blocker.27–29

Compounds 1–5 were identified as BBIQA derivatives. The BBIQAs represent a large and important class of natural products. Most are formed via phenol oxidative coupling of two N-methylcoclaurine (or cocaine) units, while a much smaller number of benzyltetrahydroisoquinoline alkaloids originate via phenol oxidative coupling of an N-methylcoclaurine and a reticuline unit. In all instances, the original bridging occurs by an ether bridge, a biaryl bond, or a methylenoxy bridge depending upon the biosynthetic route.26

The difference between seco (1) and cyclic forms (2–5) is evidenced by their 1H NMR spectra. In the seco form the protons of the 1,4-disubstituted aromatic moiety shows the expected AA’XX’ spin system, whereas in the cyclic form the four aromatic protons give separate signals caused by hindered rotation of the aryl residue. Furthermore, in the seco form H-1 and H-1’ have similar chemical shifts, but in the cyclized structures they have vastly different chemical shifts. Additionally, in the cyclic form (2 and reference d-tubocurarine) H-8 is strongly shielded and resonates near δH 5.0.

BBIQA 1 has the same molecular formula as the known BBIQA lindoldhamine.27–29

![Figure 2. Structure of lindoldhamine.](image)

Lindoldhamine was isolated from the first time from the leaves of Lindera oldhamii Hemsli. (Lauraceae)27–29 and thereafter also from Polyaltheia nitidissima (Dunal) Benth. (Annonaceae).30 Triclisia saulexii (Pierre) Diels (Menispermaceae),31 Albertsitia papuana Becc (Menispermaceae),32 Guatteria gaumeri (Mexican Annonaceae),33 Xylopia parviflora (A. Rich.) Benth. (Annonaceae),34 and Abuta pahni (Mart.) Krugoff & Barneby (Menispermaceae).35

The structure of lindoldhamine was established as a tail-to-tail bisbenzyltetrahydroisoquinoline by spectroscopic data and chemical degradations.27–29 However, there are no X-ray diffraction data and only limited information about 1H NMR data in pyridine-d5, MS, and physical properties. Our isolated compound 1 has the same molecular formula as lindoldhamine determined by HRESIMS (m/z 569.26475 [M + H]+ (calcd for C34H37N2O6, 569.26461)), but has different solubility and specific rotation ([α]D + 35 (c 0.1, EtOH),28,29 [α]D + 15.22 (c 0.3, MeOH)).

The 1H NMR data of lindoldhamine from different reports are summarized together with our own results for compound 1 in methanol-d4 in Table 1. Compound 1 is insoluble in CDCl3. Thus, it was not possible to compare our 1H NMR spectra directly with literature data of lindoldhamine.27–29,31,35,36

13C(APT), edited HSQC, and HMBC spectra of BBIQA 1 displayed 34 carbon resonances, assignable to seven aromatic
tertiary carbons, six aromatic quaternary carbons, 11 aromatic methines, two aliphatic methines, six aliphatic methylenes, and two OCH3 groups connected to aromatic rings. The NMR data featured a 1,2,4-trisubstituted and a 1,4-disubstituted benzene moiety [H-10’ (δH 6.80), H-13’ (δH 6.98), H-14’ (δH 7.02) and H-13 (δH 6.94), H-14 (δH 7.28), J = 8.1 Hz AA’XX’ spin system] together with four aromatic proton singlets at δH 6.79, 6.74, 6.61, and 6.50 (Table 2).

The HMBC spectrum shows only the expected C–H correlations via two and three bonds within the two tetrahydroisoquinoline units, but gives no additional information about the connectivity of the two moieties (tail-to-tail, head-to-head, or head-to-tail); no correlations via four bonds were observed; Figure 3b).

This was also supported by the NOESY spectrum [cross correlations between H-1 (δH 4.65), H-8 (δH 6.61), H-15 (δH 3.08/3.44 br), and H-1’ (δH 4.60), H-8’ (δH 6.50) and H-15’ (δH 3.10/3.27 br), respectively; Figure 3a]. Collectively these data indicate that BBBQQA 1 is most likely an isomer of lindoldamine with head-to-tail bonding corresponding to d-tubocurarine. It is known that, at least in Berbers, the biosynthetic formation of tail-to-tail dimers is not selective.37,38 Therefore, it cannot be ruled out that 1 may be an isomer of lindoldamine.

The HRESIMS data of BBBQQA 2 give the molecular ion [M + H]+ at m/z 595.28010, corresponding to a molecular formula of C_{36}H_{39}N_{2}O_{7}^+ (calculated at 595.28026). In contrast to 1, compound 2 exists in the cyclic form, indicated by the appearance of separated signals for the protons and the corresponding carbons of the 1,4-disubstituted aromatic ring, caused by steric hindrance. Additionally, H-8 is strongly shielded as in d-tubocurarine (δH 5.31; d-tubocurarine: δH 5.05). A major difference with regard to d-tubocurarine is the absence of one N-methyl group and the fact that both methoxy groups at C-12’ (δC 150.90) and C-6’ (δC 149.87) are part of one BBBQQA unit as determined by HMBC.

Table 1. 1H NMR Literature Data of Lindoldamine in Comparison to Compound 1

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<td>6.74</td>
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<td>3.85 s</td>
<td>3.82 s</td>
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</tbody>
</table>

A molecular formula of C_{36}H_{39}N_{2}O_{7}^+ was assigned to BBBQQA 3 on the basis of the HRESIMS ion at m/z 567.24980 [M + H]+ (calculated at 567.24896), which is consistent with a BBBQQA containing one aryl ether bridge and two N–H groups. The existence of the cyclic form is evident from the NMR data, because H-10, H-11, H-13, and H-14 and the corresponding carbons of the p-substituted aromatic ring (C-10, C-11, C-13, C-14) appear as separated signals. Most importantly, the strongly shielded proton H-8 is absent. This was confirmed by APT and edited HSQC spectra. Only nine aromatic methine protons were observed. Correlation peaks of the two aromatic proton singlets at δH 6.84 and 6.86 and the corresponding carbon atoms in the HMBC spectrum are indicative of the presence of these protons in the tetrahydroisoquinoline units and the pentasubstitution of their aromatic rings. HMBC correlations of H-1 (δH 4.87) to C-8 (δC 123.89) and H-10’ (δH 6.00) to C-8 (δC 123.89), respectively, are indicative of the unusual C–C connectivity of the two tetrahydroisoquinoline moieties.

The ECD spectrum shows two high-amplitude negative Cotton effects at 203 nm (Δε ≈ −3.56 mdeg) and 239 nm (Δε ≈ −2.84 mdeg) and high-amplitude positive Cotton effects at 216 nm (Δε ≈ +2.89 mdeg), 254 nm (Δε ≈ +2.61 mdeg), and 287 nm (Δε ≈ +2.16 mdeg) (Figure S55, Supporting Information). There are no ECD data of closely related compounds available in the literature.

BBQQA 4 represents also a new bisbenzyltetrahydroisoquinoline alkaloid, as shown by its UV and IR absorptions and NMR data. The molecular formula of C_{36}H_{39}N_{2}O_{7}^+ was assigned to compound 4 on the basis of the HRESIMS ion at m/z 569.25941 [M + H]+ (calculated 569.25953), consistent with BBQQAs containing two aryl ether bridges, one N–H, and one N-acetyl group (δC 172.64 CO, δH 2.18 s, δC 22.57 CH3). The presence of the cyclic form is evident from the NMR data, because the NMR signals of the phenyl moieties, similar to those of compounds 2–5 and d-tubocurarine, are separated due to hindered rotation.

BBQQA 5, with a molecular formula of C_{36}H_{39}N_{2}O_{7}^+ , based on the HRESIMS ion at m/z 623.27507 [M + H]+ (calculated at 623.27518), is consistent with a BBBQQA containing two aryl ether bridges, one N–CH3 (δH 2.91 br, δC 41.50 – CH3), and one N-acetyl group (δC 172.64 CO, δH 2.18 s, δC 22.57 CH3).

1H and 13C NMR data are similar to those of BBIQA 5, with differences involving the presence of the N-methyl group.

Some years ago a similar N-acetylated BBQQA was isolated from the root bark of Tiliacora racemosa Celebr (Menisperma-ceae). This plant is regarded as an antidote for snake bite.39,40 Interestingly, a derivative related to compounds 4 and 5 was reported as a blocker of L-type calcium channels.41

To evaluate the muscle relaxant activity of the Matis dart poison and its BBBQQA compounds 1–3, we conducted electrophysiology experiments. At 1 mg/mL, Matis poison reduced the acetylcholine-evoked current by 95 ± 2%, indicating that the poison contains components with muscle relaxant activity. To establish whether the muscle-relaxant properties of the Matis poison are due to the identified compounds, we performed additional electrophysiology experiments with BBBQQA compounds 1–3. These compounds share structures similar to the well-known muscle relaxant d-tubocurarine. BBBQQA compounds 1–3 reduced the acetylcholine-evoked current at 20 μM by 45 ± 8%, 88 ± 5%, and 86 ± 5%, respectively.

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Because the in vivo acute toxicity experiments indicated breathing suppression and the electrophysiology experiments showed that the Matis poison components are blockers of muscle nAChR, it seems reasonable that the toxicity of the Matis poison is associated with d-tubocurarine-like muscle relaxants. These results show for the first time that an isomer of lindoldhamine (1) inhibits muscle nAChR.

**EXPERIMENTAL SECTION**

**General Experimental Procedures.** Melting points were measured with a Büchi Melting Point B-540. Optical rotation data were obtained with a Schmidt + Haensch Polartronic MHz-8 at the sodium-D line (589 nm) using a 50 mm path-length cell with the solvent and concentration indicated. The ECD spectrum was recorded with a Jasco J-715 spectropolarimeter, and UV–vis spectra were recorded with a JASCO V-630. IR spectra were recorded with a JASCO FT/IR-4100 instrument. NMR spectra were measured using a Varian Mercury Plus (400 MHz) and a Bruker Avance III HD (400 MHz) spectrometer, respectively. HRESIMS spectra were generated with a Bruker Daltonics instrument in the ESI positive mode. Silica gel 60 (0.040–0.063 mm, 230–400 mesh, Sigma–Aldrich) were used for layer chromatography. TLC was performed on analytical aluminum sheets (H₂O/HOAc/CH₃OH and H₂O/HCl/CH₃OH) and, for preparative work, a 20 × 20 cm RP-18 F254S glass plate (Merck KGaA, 64271 Darmstadt, Germany). The chemicals were purchased from ABCR, Acros, Aldrich, Alfa Aesar, Fluorochem, TLC Europe, and VWR at the highest commercially available purity.
Poison Collection. The Matis poison was obtained in Leticia, at the Colombian–Brazilian border, by Dr. Rainer W. Bussmann. The material was authenticated and deposited at the William L. Brown Center, Missouri Botanical Garden, in November 2011. Powdered material was maintained at room temperature (22–25 °C) and protected from light until required for extraction and analysis.

Extraction and Isolation. Dried poison (5.29 g) was treated for 1 h with (100 mL) H2O using ultrasound. The water suspension was dissolved with 2% aqueous HOAc (100 mL) for 5 h to produce an acidic solution. On the surface of the solution, small pieces of bark, stem, and leaves were present. Those solids were separated by filtration through Whatmann No. 1 filter paper to yield fraction A (201.4 mg). The acidic solution was extracted several times with Et2O (100 mL portions) in order to remove nonalkaloidal compounds, wax, fat, and impurities until the acidic phase was slightly brown colored. The Et2O fraction (fraction B) was dried using Na2SO4 (about 5 to 10 g of Na2SO4 for each 100 mL), filtered, and concentrated under reduced pressure at 40 °C. The residue was afforded in a nonalkaloidal fraction (343.5 mg; negative test with Dragendorff’s reagent), containing D-(−)-quinic acid, L-malic acid, and other unidentified organic material.

Afterward the acidic solution was basified to pH 7 with gradual addition of a 25% ammonia solution under stirring. After the neutralization, the warm solution was allowed to stand for 2 h. The basic solution was extracted several times with 100 mL of CH2Cl2, CHCl3, and EtOAc. The organic layers were combined, dried with Na2SO4, filtered, and concentrated to dryness under vacuum using a rotary evaporator (40 °C), to yield 3.89 g of an alkaloid fraction (fraction C; brown oil; positive test with Dragendorff’s reagent).

Scheme 1. Two-Electrode Voltage-Clamp Measurement of the Effects of Matis Poison and Individual BBIQA Compounds 1−3 on Xenopus Oocytes Expressing Rat Muscle nAChR

"Control acetylcholine-evoked responses are shown on the right of each graph; the left part of each graph represents response inhibited by Matis poison or isolated BBIQA compounds 1−3. Matis poison applied at 1 mg/mL for 5 min reduced acetylcholine-evoked current through muscle nAChR by 95 ± 2%. BBIQA compounds 1−3 at 20 μM reduced acetylcholine-evoked current by 45 ± 8%, 88 ± 5%, and 86 ± 5%, respectively. Black bars represent 20 μM acetylcholine application.

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Silica gel 60 (40 g; Fluka) was used to separate fraction B by column chromatography with CH₂Cl₂/CH₃OH (10:1), yielding n-(−)-quinic acid (12.3 mg) and L-malic acid (3.4 mg). Fractions 4−9 (26.2 mg; 1.31%), and BBIQA 3 (16.5 mg; 0.82%). After RPC of fractions 4−9, a dark green mixture was obtained. Using RPC (elucent 1.0 M HCl) to remove the dark green color afforded the corresponding fraction 10.

Fraction 10 (110.0 mg) was eluted [RPC with H₂O/HCl/CH₃OH (50:0.1:50 to 0:1:1:99.9:1); 2.5 M HCl and purified by preparative RP-TLC; H₂O/HCl/CH₃OH (60:0:1:40) to afford BBIQA 4 (10.1 mg; 0.51%) and BBIQA 5 (15.0 mg; 0.75%).

**Table 3. NMR Spectroscopic Data of Compounds 4 and 5 and d-Tubocurarine Recorded in Methanol-d₄ at 400 MHz for ¹H and 100 MHz for ¹³C (δ in ppm)**

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<td>2.95 br</td>
</tr>
<tr>
<td>6-OCH₃</td>
<td>3.86 s</td>
<td>56.77 CH₃</td>
<td>3.78 s</td>
</tr>
<tr>
<td>1′</td>
<td>5.94 d (9.7)</td>
<td>52.81 CH</td>
<td>5.93 d (10.9, 1.5)</td>
</tr>
<tr>
<td>3α′</td>
<td>3.32 br</td>
<td>45.88 CH₂</td>
<td>3.32 br</td>
</tr>
<tr>
<td>3β′</td>
<td>6.56 dd (8.2, 2.2)</td>
<td>130.37 CH</td>
<td>7.37 dd (8.6, 2.2)</td>
</tr>
<tr>
<td>4α′</td>
<td>2.95 br</td>
<td>21.36 CH</td>
<td>2.95 br</td>
</tr>
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<td>4β′</td>
<td>3.32 br</td>
<td>3.31 CH</td>
<td>3.32 br</td>
</tr>
<tr>
<td>5′</td>
<td>6.84 s</td>
<td>108.75 CH</td>
<td>6.84 s</td>
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<tr>
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<td>149.31 C</td>
<td>149.31 C</td>
</tr>
<tr>
<td>7′</td>
<td>138.26 C</td>
<td>138.27 C</td>
<td>138.26 C</td>
</tr>
<tr>
<td>8′</td>
<td>139.25 C</td>
<td>139.22 C</td>
<td>139.25 C</td>
</tr>
<tr>
<td>9′</td>
<td>124.15 C</td>
<td>124.14 C</td>
<td>124.15 C</td>
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<tr>
<td>10′</td>
<td>132.06 C</td>
<td>132.14 C</td>
<td>132.06 C</td>
</tr>
<tr>
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<td>6.56 dd (8.5, 2.2)</td>
<td>118.55 CH</td>
<td>6.56 dd (8.6, 2.8)</td>
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<tr>
<td>12′</td>
<td>155.43 C</td>
<td>155.27 C</td>
<td>155.43 C</td>
</tr>
<tr>
<td>13′</td>
<td>5.81 dd (8.2, 2.5)</td>
<td>114.90 CH</td>
<td>5.80 d (8.0)</td>
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<tr>
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<td>6.21 dd (8.2, 2.2)</td>
<td>129.33 CH</td>
<td>6.21 dd (8.4, 2.2)</td>
</tr>
<tr>
<td>2 x 2′-NCH₃</td>
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<td>172.64 CO</td>
<td>2.18 s</td>
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<tr>
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<td>2.27 CH₃</td>
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<tr>
<td>6′-OCH₃/12′-OCH₃</td>
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<td>56.77 CH₃</td>
<td>3.86 s</td>
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<tr>
<td>15′/5χβ</td>
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<td>2.88/3.44 br</td>
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<td>2.68/3.04 br</td>
<td>44.72 CH₂</td>
<td>2.69 m/3.04 br</td>
</tr>
</tbody>
</table>

*Signal peak broadening by hindered rotation: not all the coupling constants could be accurately determined.*
**REFERENCES**

(42) http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm (12.08.2015).