Nigericic Acids, Two New Chemical Derivatives of Nigericin

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Carboxylic polyether antibiotics belong to a well-documented ionophore family,1) and several components are currently used as anticoccidal compounds for poultry or to improve the efficiency of feed utilization in ruminant animals. In the mycelium cake harvested from a culture of Streptomyces hygroscopicus NRRL B 1865, producing nigericin2) (1), a new polyether antibiotic (abierixin) was found.3) The structures of these two antibiotics are almost similar, the only difference being the opening of the A ring for abierixin.4) According to the model of nigericin biosynthesis proposed by Cane,5) abierixin is similar to the ultimate metabolic precursor of nigericin, which gives nigericin by a Michael reaction. In nigericin, two trans-bulky groups on C3 and C7 might destabilize the A ring. Thus, abierixin might be an artefact due to the growth conditions (basic pH) or to the extraction conditions (acidic pH), and result from the opening of the A ring of nigericin by a retro-Michael reaction. To check the latter hypothesis, nigericin was treated under both acidic and basic conditions. Nigericin treated by HC1 (1 N in an aqueous alcoholic mixture [50:50]) was transformed into several products, with a reaction yield of 10%. From the Rf values, it was concluded that none of these products was abierixin. In basic conditions, nigericin was transformed into nigericic acid (2, 80%) and isonigericic acid (3, 20%). A solution of nigericin (500 mg) in a 250 ml aqueous alcoholic mixture (50:50, v/v) was adjusted to pH 11 with NaOH. The mixture was stirred with aeration for 24 hr and the transformation was followed by TLC (silica gel 60F-204 (Merk); solvent CHCl3-MeOH, 90:10). The products were extracted with CHCl3, and separation of nigericic acid and isonigericic acid was achieved by flash column chromatography (CHCl3-MeOH with an increasing amount of MeOH as the solvent, starting from a 95:5 mixture). The reaction yield was 60% (nigericic acid, Rf=0.35 (CHCl3-MeOH, 90:10), [α]25°=−6.6°, mp=136°C; isonigericic acid, Rf=0.43, [α]25°=+7.3°, mp=124°C).

The structures of nigericic acids (2) and (3) (Fig. 1) were determined by analyses of their IR, MS and NMR spectra. The IR spectra of (2) and (3) (KBr) showed absorption at 3500-3200 cm⁻¹, 1700 cm⁻¹ and 1100-1020 cm⁻¹, corresponding to ν OH, ν COOH and ν C–O–C, respectively. The FAB-MS spectra of (2) and (3) were very similar. For both compounds, the (M−H)⁻ ion observed in FAB-MS (negative ionization) at m/z 739 corresponded to the suggested molecular formula C₄₀H₆₈O₁₂. A weaker peak was observed at m/z 763 corresponding to the sodium complex of nigericic acids (M+Na)⁺. This was the most prominent in the FAB-MS (positive ionization). The fragment at m/z 763 corresponding to the fragmentation a (Fig. 1) easily lost 2H₂O and CO₂ to give the fragment m/z=95. Furthermore, the fragmentation proposed by Siegel⁵ corresponding to the A, B, C, D and E rings of nigericin can be found. Complete assignments of the ¹H and ¹³C NMR spectra of (2) and (3) were performed by ¹H–¹H and ¹H–¹³C chemical shift correlations,⁶ and the ¹³C parity was determined by a J modulated spin echo experiment. The general strategy for the assignment was similar to that used for nigericin. ⁷ 

![Fig. 1. Structures of Nigericic Acid and Isonigericic Acids 2 and 3.](image)

Characteristic FAB-MS fragments.
described previously in the case of the ionophorous antibiotic X14547A.\textsuperscript{7} The \textsuperscript{1}H and \textsuperscript{13}C chemical shift comparison of the A, B, C, D and E rings of (2) and (3) showed great similarity with the corresponding nigericin rings. A new proton appeared on C29 in (2) and (3) (\(\delta=4.20\) (d, \(J=2\) Hz) and 4.15 (d, \(J=2\) Hz), respectively), and the methylene protons of C30 (CH\textsubscript{2}OH) disappeared. The COSY spectra of (2) and (3) showed connectivity of \textsuperscript{1}H: 24, 25, 26, 32, 27 (A, B), 28, 31 and 29. The \textsuperscript{13}C NMR spectra of (2) and (3) showed the presence of two CO\textsubscript{2} and the disappearance of the methylene carbon (C30, CH\textsubscript{2}OH). Furthermore, the hemiketalic quaternary carbon C29 (\(\delta=97.2\)) was replaced by a tertiary carbon (\(\delta=\) 78.8 or 74.8). The determination of the configuration of C29 and the confirmation of the structure were achieved by chemical correlation. Nigericin was reduced by LiAlH\textsubscript{4} to two alcohols, (6, 29S) and (7, 29R). In the presence of NaBH\textsubscript{4}, nigericin was reduced to two acid alcohols, (4, 29S, 30\%) and (5, 29R, 70\%). Their stereochemistries at the C29 position were determined previously.\textsuperscript{8} The reduction by LiAlH\textsubscript{4} of (3) and (4) on one hand, and of (2) and (5) on the other hand gave alcohols (6) and (7). This chemical correlation allowed us to conclude that the C29 absolute configurations of nigericic acid (2) and isonigeric acid (3) are R and S, respectively.

Nigericic acids might be formed by a mechanism of ketolic transposition after the opening of the nigericin F ring, and followed by oxidation of the aldehyde in the presence of air oxygen \((-\text{C}(=\text{O})-\text{CH}_2\text{OH} \rightarrow -\text{C(OH)} = \text{CHOH} \rightarrow -\text{CH(OH)} \rightarrow -\text{CHO})\).

The integrity of the terminal hemiketalic function is essential for the ionophore properties of carboxylic polyether antibiotics.\textsuperscript{9} The experiments performed previously on (4) and (5)\textsuperscript{9} had shown an important decrease of the antibiotic properties of these products. Our purpose was to confirm this observation by measuring the antibiotic and complexing properties of the nigericic acids. The antibiotic properties of (2) and (3) were compared with those of nigericin, using the conventional dilution method with \textit{Bacillus cereus} ATCC 14579 in Mueller-Hinton broth at pH 7. (2) and (3) were weak antibiotics, their MIC values being identical with those observed for the corresponding alcohols (4) and (5) at 0.25 mg/l (0.05 mg/l for nigericin). The complexing properties of (2) were also tested by using a biphasic extraction system\textsuperscript{10} and were lost for sodium and potassium. These results confirm that the antibiotic activity was linked to the complexing properties of the ionophores, and demonstrate that the loss of the hemiketal group strongly decreases these properties. The anticoccidial evaluation of (2) was carried out on a 17-day-old chicken with \textit{Tenericia tenella} oocysts at the Ploufragan experimental station, the test being continued for eight days. At 80 ppm, (2) showed a weak anticoccidial activity.

In this paper, we have shown that abierixin was not an artefact due to the growth or extraction conditions of nigericin. The nigericic acids were isolated and their precise configuration was determined by using a chemical correlation. The opening of the terminal F ring observed for nigericic acids (2) and (3) induced the loss of the antibiotic and complexing properties. This confirms our previous results obtained with the same type of molecule\textsuperscript{8} for (4) and (5).

References