A NEW SOURCE OF KOJIC ACID ISOLATED FROM KIGELIA AFRICANA: A POSSIBLE PRECURSOR FOR QUINONE BIOSYNTHESIS

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ABSTRACT
Kojic acid (5-hydroxy-2-hydroxymethyl-γ-pyrone) a fungal metabolite produced by Aspergillus spp., Penicillium spp. and belonging mainly to the flavus-oryzaetamarii groups was isolated for the first time from Kigelia africana as the major constituent by mass fragmentation guided isolation. From a biosynthetic consideration, Kojic acid is a possible intermediate in the synthesis of the quinone scaffolds.

Keywords: Kigelia africana, Bignoniaceae, Quinones, Kojic acid, Biosynthesis.

INTRODUCTION
Plants have a long history of use on the African continent for the treatment of different diseases and complaints. In certain African countries, up to 90% of the population still relies exclusively on plants as a source of medicines. Africa is reputed for the extraordinary richness of its flora, totaling several tens of thousands of species. As part of our systematic search for new bioactive lead structures from African medicinal plants, Kigelia africana was selected for chemical and biological investigations. Previous phytochemical studies carried out on Kigelia africana have led to the isolation of mainly quinones, iridoids, fatty acids, norviburtinas, sterols, lignans, terpenoids, isocoumarins and show antidiarrhoea, antileprotic, antimalarial, anti-inflammatory, anticancer, gynaecological disorders, anti-implantation, Central Nervous System stimulant, antimicrobial and miscellaneous medicinal properties.

EXPERIMENTAL
General Procedure
NMR spectral data were measured with a Bruker Avance DRX 500 NMR, HR-MS spectra were recorded on a LTQ Orbitrap Spectrometer equipped with an APCI ion source (Ion Max) operating in positive mode. Column and Flash chromatography was performed on silica gel (60-120 mesh, Merck). Analytical TLC was performed on Merck silicagel 60F254 pre-coated Aluminum plates.

Plant materials: The root barks of Kigelia africana were collected in Limbe near the Mount Cameroon area (South West region of Cameroon) on the 30th of June 2009. The plant was identified with the help of Mr Ndive Elias an ethnobotanist. A voucher specimen, N° HNC-157, has been deposited at the Yaounde National Herbarium.

Extraction and isolation
Dried and powdered root bark (5 kg) of kigelia africana was separately extracted with a mixture of MeOH: CH₂Cl₂ (1:1) at room temperature for 48 h. The suspensions were filtered and each filtrate was concentrated under suction to give 400 g of crude extract. The crude extract was then subjected to flash chromatography using hexane, hexane-ethyl acetate 10%, hexane-ethyl acetate 25%, hexane-ethyl acetate 50%, ethyl acetate 100% and methanol 100% to afford 3g, 6g, 12g, 10g, 20g and 30g fractions.
respectively. The Methanol fraction was fixed with 55 g of silica gel and eluted over silica gel (G 60 Merck) with a gradient mixture of hexane, ethyl acetate and methanol. 125 fractions (F1-125) were collected each with a volume of 130 mL. Fractions F84-87 from elution with a mixture of EtOAc:MeOH (95:5) yielded kojic acid (1) (95 mg). After evaporation a crystal with the dimensions of 0.30 mm x 0.40 mm x 0.60 mm was obtained. It was mounted on a Mitegen Micromount and automatically centered on a Bruker SMART X2S benchtop crystallographic system. Intensity measurements were performed using monochromated (doubly curved silicon crystal) Mo-Kα-radiation (0.71073 Å) from a sealed microfocus tube. Generator settings were 50 kV, 1 mA. Data collection temperature was 27°C.

Kojic acid
It was obtained as white crystals and melted at 151°C. Its HPLC-UV-MS spectrum showed a base peak [M + H]+ at m/z 143.03383 suggesting a molecular formula of C₆H₇O₄. ¹H-NMR (CD₃OD, 500 MHz): 7.95 (s, 1H), 6.50 (s, 1H), 4.41 (s, 2H), 3.35 (s, OH). ¹³C-NMR (CD₃OD, 125 MHz): 176.7, 170.2, 147.2, 140.8, 110.6 and 61.1.

RESULTS AND DISCUSSION
To investigate miscellaneous medicinal properties, dried and powdered root bark (5 kg) of kigelia africana was separately extracted with a mixture of MeOH: CH₂Cl₂ (1:1) at room temperature for 48 h. The suspensions were filtered and each filtrate was concentrated under suction to give 400 g of crude extract. High-Resolution mass spectra (HR-MS) of the crude extract were recorded using a LTQ Orbitrap Spectrometer equipped with an APCI ion source (Ion Max) operating in positive mode. The spectrometer was equipped with a surveyor HPLC system consisting of LC-pump, PDA detector, and auto-sampler (injection volume 10 µL). Nitrogen was employed as both the sheath (40 arbitrary units) and auxiliary (10 arbitrary units) gas. First LC-MS screening of the extract revealed the presence of a compound with a quasimolecular ion [M + H]+ at m/z 143.03383 (theoretical value: 143.03389). This was the most abundant signal and different from previously reported compounds. The crude extract was then subjected to flash chromatography using hexane, hexane-ethyl acetate 10%, hexane-ethyl acetate 25%, hexane-ethyl acetate 50%, ethyl acetate 100% and methanol 100% to afford 3g, 6g, 12g, 10g, 20g and 30g fractions respectively. A second mass analysis of all fractions revealed the presence of the most abundant compound in the methanol fraction. Column chromatographic separation of the methanolic fraction yielded kojic acid (1) (95 mg). Kojic acid (5-hydroxy-2-hydroxymethyl-γ-pyrone) (1), a fungal metabolite produced by Aspergillus spp., Penicillium spp. and belonging mainly to the flavus-oryzaetamarii groups¹⁰-¹¹ was isolated for the first time from Kigelia africana as the major constituent by mass fragmentation guided isolation. The structure was unambiguously confirmed from single crystal structure analysis and compared with previously recorded crystallographic data¹².

Fig.-1: X-ray structure of Compound-1
From a biosynthetic consideration (scheme 2), Kojic acid is a possible intermediate in the shikimic acid pathway. This pathway involves the condensation of phosphoenol pyruvate (C₃ unit) (3) with a tetrose (D-erythro-4-phosphate) (4) to yield 3-deoxy-D-arabinoheptulosonic acid (DAHP) (5), a seven carbon sugar which can be converted to shikimic acid (2)¹³-¹⁴ or undergoes intramolecular condensation and subsequent dehydration and oxidation to kojic acid (1) (scheme 2). Since shikimic acid (2) is a key
intermediate in the biosynthesis of quinones, Kojic acid can also be considered as a possible precursor in the biogenesis of the quinone scaffold. Further investigation is however needed for the conversion of Kojic acid to quinones.

Scheme-1: Previously isolated compounds from *Kigelia africana*
ACKNOWLEDGEMENTS

Dr. K.O Eyong thanks DAAD Germany for financial support and a research stay at the Technical University of Dortmund. The authors are grateful to Bruker AXS (Karlsruhe, Germany) for the X-ray measurement.

REFERENCES


[RJC-989/2012]