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CO-OCCURRENCE OF 3,3′,4′-TRI-*O*-METHYLFLAVELLAGIC ACID AND 3,3′-DI-*O*-METHYLELLAGIC ACID IN THE BARK OF *ANOGEISSUS SCHIMPERII*

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Key Word Index—*Anogeissus schimperii*, Combretaceae, 3,3′,4′-tri-*O*-methylflavellagic acid, 3,3′-di-*O*-methylellagic acid, tri-*O*-methylellagic acid

Abstract—The first isolation of 3,3′,4′-tri-*O*-methylflavellagic acid from a natural source and its co-occurrence with 3,3′-di-*O*-methylellagic acid and tri-*O*-methylellagic acid in the bark of *Anogeissus schimperii* is described. Its structure was established on spectroscopic evidence

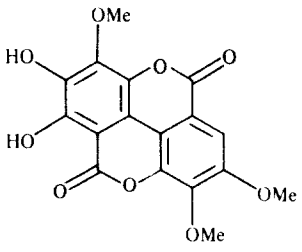
INTRODUCTION

Anogeissus schimperii grows wild in Nigeria along the Savanna belts. The leaves are used by traditional tanners in the northern parts of Nigeria for making yellow crust leathers [1] Previous chemical examinations yielded some polyols and glucuronic acid derivatives from the gum exudates of the bark [2, 3], benzoic acids and ellagic acid from the leaves and ellagitannin constituents from the sapwood [4, 5]

In a continued phytochemical study of this potential tanning plant, more polyphenols have been identified in the bark. We now wish to report the first isolation of 3,3′,4′-tri-*O*-methylflavellagic acid **1**, from a natural source and its co-occurrence with 3,3′-di-*O*-methylellagic acid **2**, and a tri-*O*-methylellagic acid

RESULTS AND DISCUSSION

3,3′,4′-Tri-*O*-methylflavellagic acid **1**, was obtained as a precipitate from aqueous acetone extract of the bark Recrystallization from pyridine–DMF mixture (7 3) gave yellow flakes, mp 292–295° (lit [6] mp 300–302°) The IR spectrum showed an absorption at 1700 cm^{-1} due to carbonyl group of an aryl lactone The absorption at 3275 cm^{-1} is due to OH group characteristic of phenols [7]. The 90 MHz NMR spectrum in DMSO-d_6 indicated the presence of three methoxyl groups at δ 4 1, 4 0 and 3.9 and an aromatic proton signal at δ7 5 It analysed for $C_{17}H_{12}O_9$ (M^+ 360) suggestive of a trimethyl ether of flavellagic acid [6]. The position of the two free hydroxyl groups were decided by UV measurements in ethanol and in the presence of shift reagents (Table 1) The bathochromic shift of 46 nm of the long wavelength band in EtOH + NaOAc was associated with the presence of a free OH group at C-4 and the protection of the OH groups at C-3 and C-3′ in agreement with other workers [6, 8]. The case for a free OH group at C-4 was strengthened by the fact that the short wavelength band was unaffected in the presence of NaOAc [9] This is also in accordance with an earlier observation [6] for synthetic 3,3′,4′-tri-*O*-methylflavellagic acid However, no significant bathochromic shift was observed at the long wavelength band on addition of NaOEt (λ_{max} 368 nm) indicating the absence of a 4,4′-dihydroxy system [9] There was also no significant shift at the long wavelength band when the reagent was changed from NaOEt to NaOAc + H_3BO_3 as was observed for *o*-dihydroxy systems in flavonoids [10]. This is suggestive of the absence of a 4,4′-dihydroxy system and thus confirmed that the hydroxyl at C-4 was protected Also, a large bathochromic shift of the long wavelength band was recorded (45 nm) in the presence of $AlCl_3$, indicative of



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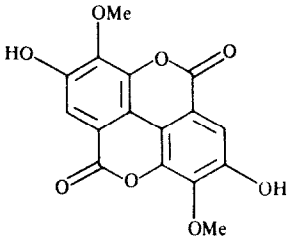

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Table 1 UV spectral data for 3,3',4'-tri-*O*-methylflavellagic acid

Compound	λ_{max}(nm)	λ_{max}(nm)
3,3',4'-Tri-*O*-methylflavellagic acid in EtOH	245 58	367 00
+ NaOAc	245 75	413 00
+ NaOAc + H_3BO_3	282 80	368 00
+ $AlCl_3$	245 58	412 00
+ NaOEt	–	368 00

Table 2 UV spectral data for 3,3'-di-*O*-methylellagic acid

Compound	λ_{max}(nm)	λ_{max}(nm)
3,3'-Di-*O*-methylellagic acid (in EtOH)	248 22	375 72
+ NaOAc	259 75	413 92
+ NaOAc + H_3BO_3	289 00	380 00
+ $AlCl_3$	248 92	375 58
+ NaOEt	245 58	375 58

the presence of a free 5-hydroxyl group in the tri-methylated flavellagic acid forming a metal complex with the neighbouring carbonyl group typical of the free 3-hydroxyl group in flavonoids [6]. These spectroscopic properties are in good agreement with structure **1** which has been established for synthetic 3,3',4'-tri-*O*-methylflavellagic acid [6]. This is the first report of the isolation of 3,3',4'-tri-*O*-methylflavellagic acid from a natural source. Hitherto, this derivative was prepared by synthesis only [6]

3,3'-Di-*O*-methylellagic acid **2**, was also obtained as a fraction of the precipitate from the aqueous acetone extract of the bark of *A. schimperii*. It crystallized from pyridine as yellow flakes, mp 325–327° (lit. [6] mp 325–327°). The IR spectrum showed absorption at 1730 cm^{-1} suggestive of the presence of carbonyl group conjugated with an aromatic ring. This is supported by the absorptions at 1690 and 1600 cm^{-1} which are indicative of aryl lactone system. The absorption at 3400–3200 cm^{-1} is suggestive of hydrogen bonding due to free OH group. The ^{1}H NMR of the compound in DMSO-d_6 showed aromatic proton signal at δ7 5 (2H) and two methoxy protons at δ4.0 (6H) supportive of di-*O*-methylellagic acid system. The compound analysed for $C_{16}H_{10}O_8$ (M^+ 330). UV measurements (Table 2) in EtOH; (+NaOAc); (+NaOEt); (+NaOAc+H_3BO_3) were in good agreement with literature values for 3,3'-di-*O*-methylellagic acid [8].

The MS analysis of **2** also showed a peak at *m/z* 344 which is suggestive of the presence of a tri-*O*-methylellagic acid as an impurity. This work therefore records the co-occurrence of 3,3',4'-tri-*O*-methylflavellagic acid, 3,3'-di-*O*-methylellagic acid and a tri-*O*-methylellagic acid in the bark of *A. schimperii*, compounds which are probably associated with the tanning/filling characteristics of the plant in leather technology. In combination with previous reports on the isolation of gallic, gentisic and ellagic acids from *A schimperii* [4, 5], this work lends strong support to a biogenetic pathway of successive hydroxylation and methoxylation processes for polyphenols and their derivatives in plants [11].

EXPERIMENTAL

Mps uncorr ^{1}H NMR. 90 MHz (TMS as int. reference) MS. (EI) at 70 eV.

Plant material The bark of *A schimperii* was collected from Zaria, in the northern part of Nigeria in May 1983 Voucher specimens were confirmed and deposited at the Savanna Forestry Research Station, Samaru, Zaria It was air-dried and reduced to a fine powder

Extraction and isolation of compounds

3,3',4'-*Tri*-O-*methylflavellagic acid* **1** Powdered bark (2 kg) was extracted with 90% aq acetone (3 × 5 l) The combined extract was filtered and allowed to stand overnight at room temp A light yellow solid pptd and filtered off, washed several times with H_2O and then stirred with 70% aq acetone (3 × 50 ml). Filtration followed by evapn of the filtrate to dryness gave 3,3',4'-tri-*O*-methylflavellagic acid **1** which crystallized from pyridine–DMF (7 3) as yellow flakes, 55 mg, mp 292–295° (lit [6] mp 300–302°) λ_{max}^{EtOH} 246, 367, (+NaOAc) 246, 413, (+NaOAc+H_3BO_3) 283, 368, (+$AlCl_3$) 246, 412, (+NaOEt) 368, (+NaOEt+H_3BO_3) 368 nm ν_{max}^{KBr} 3275 (OH), 1700 (C=O), 1600, (Ar. C=C), 1280 (C–O–C) cm^{-1} δ(DMSO-d_6) 7 5 (1H, aromatic), 4 1 (3H, –OMe), 4 0 (3H, –OMe), 3 9 (3H, –OMe) MS *m/z* 360 (M^+) Analysis for $C_{17}H_{12}O_9$ Found C, 56 42, H, 3 49%, requires: C, 56.66, H, 3 30%

3,3'-*Di*-O-*methylellagic acid* **2** Recrystallization of the insoluble residue from pyridine gave yellow flakes, (150 mg), mp 325–327° (lit [6] mp 325–327°) λ_{max}^{EtOH} 248, 375, (+NaOAc) 259, 413, (+NaOAc+H_3BO_3) 289, 380, (+$AlCl_3$) 249, 376, (+NaOEt) 246, 376 nm ν_{max}^{KBr} 3400–3200 (OH), 1730, 1690 (C=O), 1600, 1580 (Ar C=C), 1290 (C–O–C) cm^{-1} δ(DMSO-d_6) 7.5 (2H, aromatic), 4 0 (6H, 2x-OMe) MS *m/z* 330 (M^+), 344 Analysis for $C_{16}H_{10}O_8$ Found C, 57 80, H, 3.21%, requires C, 58 20, H, 3 27%

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A NORLIGNAN, CRYPTORESINOL, FROM THE HEARTWOOD OF *CRYPTOMERIA JAPONICA*

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Key Word Index—*Cryptomeria japonica*, Taxodiaceae, heartwood phenol, norlignan; cryptoresinol.

Abstract—Cryptoresinol, a new norlignan has been isolated from the heartwood of *Cryptomeria japonica* and its structure was elucidated as 3,5-di(*p*-hydroxyphenyl)-2,5-epoxy-1-pentanol-3-en

INTRODUCTION

Japanese cedar (*Cryptomeria japonica* D. Don), commonly called sugi, grows well almost all over Japan. Its timber has been extensively used in the construction of Japanese houses.

We have now investigated extracts in the heartwood of *Cryptomeria japonica* Kai *et al.* [1–4] and ourselves [5] have isolated sugiresinol (**6**), hydroxysugiresinol (**7**), agatharesinol (**4**) and sequirin-C (**5**), called as norlignans (C_{17} phenolic compounds), which are regarded as useful markers for chemical taxonomy of Taxodiaceae. The isolation has been reported also of metasequirin (A and B), hydroxymetasequirin, athrotaxin, hydroxyathrotaxin, agatharesinol, sequirin (A–G) and hinokiresinol from Taxodiaceae [5–11].

In the course of our study, we isolated a new phenolic compound which we called compound Z [5]. We now propose to name this new phenolic compound Z as cryptoresinol. We report on its structural elucidation as a new type of norlignan having the hydrofuran ring and not a pyran ring.

RESULTS AND DISCUSSION

Cryptoresinol (**1**), isolated from the methanol extractives of the heartwood of *Cryptomeria japonica* by silica gel chromatography, had the molecular formula $C_{17}H_{16}O_4$ ([M^+] at *m/z* 284). Methylation with ethereal diazomethane afforded the dimethyether derivative (mp 91–93°) which showed [M^+] at *m/z* 312 suggesting the molecular formula as $C_{19}H_{20}O_4$. Acetylation with acetic anhydride in pyridine yielded the triacetate (oil) which showed [M^+] at *m/z* 410 suggesting the molecular formula as $C_{23}H_{22}O_7$. Thus, it was proved that among the four oxygen atoms present in cryptoresinol, two were phenolic and one was an alcoholic hydroxyl group. The residual oxygen atom must exist as the ether linkage because the IR spectrum of cryptoresinol showed no carbonyl absorption band.

The ^{1}H NMR spectrum of the triacetate revealed that the signals for the protons on aromatic rings appeared as four AB type coupling groups (4 sets of 2H, *d*, $J = 8$ Hz at δ 7.02, 7.05, 7.30 and 7.41) suggesting two *p*-hydroxyphenyl structures. The UV spectrum of cryptoresinol showed λ_{max} 265 nm (log ε 4.26) indicating a double bond involved in a styryl chromophore.

In the catalytic reduction of cryptoresinol (**1**) by 5% Pd-C in a hydrogen atmosphere, the dihydro derivative (dihydrocryptoresinol (**2**)) was formed, which was different from sugiresinol (**6**) by having a pyran ring. Further catalytic reduction of the dihydro derivative finally afforded another hydrogenolysed product, which was not crystallized but it was chromatographically and spectroscopically identical with authentic dihydroagatharesinol (**3**).