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APHANANIN, A TRITERPENE FROM *APHANAMIXIS POLYSTACHYA**

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Key Word Index—*Aphanamixis polystachya*; Meliaceae; 21,23*S*-epoxytirucall-7-ene-3β,21β,24,25-tetrol 3β-monoacetate; aphananin.

Abstract—The structure of aphananin isolated from the fruits of *Aphanamixis polystachya* was established as 21,23*S*-epoxytirucall-7-ene-3β,21β,24,25-tetrol 3β-monoacetate from spectral analyses and chemical transformations.

INTRODUCTION

The plant *Aphanamixis polystachya* Wall & Parker has been reported to yield a number of triterpenoids of diverse structural skeleta [1–8] including a diterpene alcohol [9]. Further investigation of the fruits has culminated in the isolation of a new triterpene, aphananin, bearing a hemiacetal system which may be considered as a biogenetic intermediate between butyrospermol and C_{26}-triterpenoids. The present communication is concerned with the structure elucidation of aphananin (**1**), a Δ^7-tirucallol type triterpene.

RESULTS AND DISCUSSION

The compound **1**, $C_{32}H_{52}O_6$, $[M]^+$ 532, produced a brilliant pink coloration in the Liebermann–Burchard test indicating it to be a triterpene. The IR Spectrum (KBr) exhibited strong absorption at 3380 (br, hydroxyls), 1725 and 1260 (acetate), and 818 cm^{-1} (trisubstituted double bond). The presence of the trisubstituted double bond was also indicated from the positive tetranitromethane colour reaction. The 80 MHz ^1H NMR spectrum ($CDCl_3$) showed five 3H singlets at δ0.75, 0.80, 0.85, 0.93 and 0.97 for tertiary methyl groups. Two 3H singlets at δ1.23 and 1.25 clearly indicated the presence of a gem-dimethyl group at C-25. A 3H singlet at δ2.02 characterized the presence of an acetate. Appearance of the C-3 proton at δ4.62 (J = 11 Hz) as a triplet was characteristic for a 3α-proton in 3β-triterpene acetates. A signal at δ3.73 (1H) disappearing on shaking with D_2O could be assigned to a hydroxyl function. A broad signal at δ3.24 integrating for three protons (of which two were D_2O exchangeable) was ascribed to two hydroxyl groups and the proton attached to C-24. A sharp multiplet at δ4.40 (1H) strongly suggested the presence of a methine proton in a situation like that shown in partial structure **3**. The signal at δ5.22(2H) appeared to be due to the presence of an olefinic proton at C-7 and the proton at C-21. The ^1H NMR spectral data of compound **1** were very similar to those of turraeanthin isolated from *Turraeanthus africanus* [10] and aphanamixin (**2**), isolated from *Aphanamixis polystachya* [1, 3]. However, signals at δ2.85 (H-24) and 3.86 (H-23) in aphanamixin were replaced by a signal at δ3.24 and a multiplet at 4.40, respectively, in aphananin. Moreover, the molecular ion peak at $[M]^+$ 532 was only eighteen mass units higher than that of aphanamixin. It was, therefore, reasonable to assume that in the new compound one of the epoxide rings in aphanamixin was probably opened up. This was corroborated by the smooth hydrolysis of aphanamixin (21,23*S*,24*R*,25-diepoxytirucall-7-ene-3β,21β diol 3β-monoacetate) to a diol using a standard procedure [11] which does not involve any configurational change of the molecule. The β-configuration of the hydroxyl group at C-21 in aphanamixin was, in turn, established beyond ambiguity by its correlation [4] with turraeanthin and melianone. The diol derived from aphanamixin under the above hydrolytic condition must, therefore, retain the β-configuration of the C-21 hydroxyl as in aphanamixin. The identity (mp, mmp, co-TLC, superimposable IR and ^1H NMR spectra) of the diol with aphananin established the structure of the new terpenoid as **1** (21,23*S*-epoxytirucall-7-ene-3β,21β,24,25-tetrol 3β-monoacetate). The occurrence of very related compounds viz. meliantriol and 3-epimeliantriol from *Melia azedarach* [12, 13] having a specific configuration of C-21 hydroxyl has also been reported.

The structure **1** for the new triterpene was further supported by ^{13}C NMR spectral data. The chemical shifts for the ^{13}C NMR spectrum were assigned (shown in **1a**) and the interpretations were in conformity with other similar Δ^7-tirucallol type triterpenes [14].

The downfield shift of the signal for C-23 in the ^{13}C NMR spectrum of aphananin compared to that (δ78.3) of turraeanthin [14] might be due to the opening of the 24,25-epoxide ring. The compound aphananin was present in the crude extract and aphanamixin (**2**) did not furnish any aphananin under the isolation conditions. So aphananin is believed to be a true natural product and not

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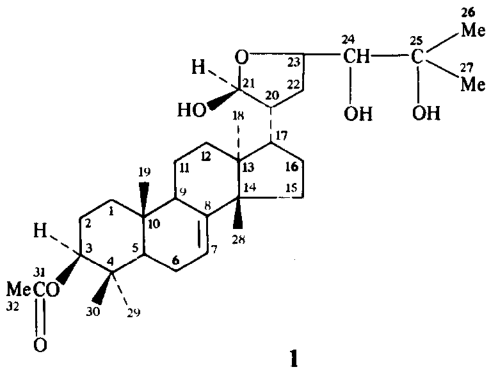

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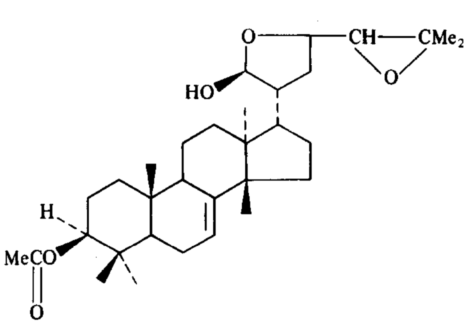

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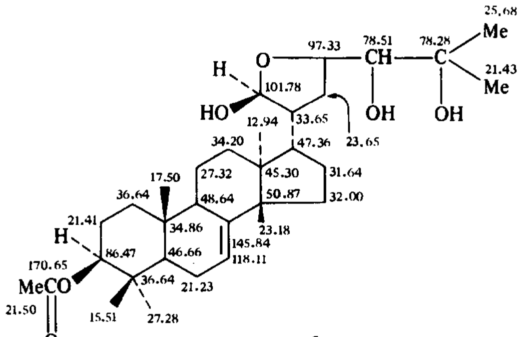
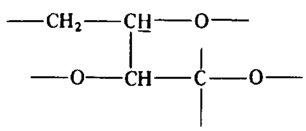


1a



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an artefact. The isolation of related compounds viz. meliantriol [12] and turraeanthin diol [15] would also substantiate this contention.

EXPERIMENTAL

Mps are uncorr. ^1H NMR (80 MHz) and ^{13}C NMR (50 MHz) spectra were recorded in $CDCl_3$ with TMS as an internal standard. The MS were recorded at 70 eV. Silica gel (BDH, 60–120 mesh) was used for column chromatography and silica gel G (Merck) for TLC. Analytical samples were routinely dried over P_2O_5 *in vacuo* for 24 hr.

Isolation of aphananin (**1**). Air-dried powdered defatted fruits (800 g) of *A. polystachya* were exhaustively extracted with acid free $CHCl_3$ in a Soxhlet for 24 hr. After removal of solvent the crude extract (58 g) was chromatographed over silica gel. The C_6H_6–EtOAc (3 : 1) eluates afforded a white solid of compound **1**. It was purified by repeated crystallization from C_6H_6–$CHCl_3$, mp 151–152°, yield: 0.0125%; R_f 0.39 (C_6H_6–EtOAc, 1 : 1); MS *m/z*: 532 $[M]^+$.

Acid hydrolysis of aphanamixin. To aphanamixin (10 mg) was added 5 ml 0.1 N H_2SO_4–THF (1 : 1) and the reaction mixture was allowed to stand at room temp for 4 hr. After usual work-up the product was recrystallized from C_6H_6–$CHCl_3$, mp 151–152°, and was found to be identical with aphananin by direct comparison (mmp, co-TLC, co-IR and ^1H NMR).

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ANTHOCYANIDIN GLYCOSIDES FROM THE FLOWERS OF *ALSTROEMERIA*

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Key Word Index—*Alstroemeria*; Alstroemeriaceae; 6-hydroxycyanidin; 6-hydroxycyanidin 3-rutinoside; 6-hydroxycyanidin 3-glucoside; cyanidin 3-rutinoside.

Abstract—Three anthocyanins were isolated from the red flowers of four cultivars of *Alstroemeria*. Two compounds were novel anthocyanidin glycosides; the 3-rutinoside and 3-monoglucoside of 6-hydroxycyanidin. Cyanidin 3-rutinoside was also present in the flower petals.

INTRODUCTION

The flowers of *Alstroemeria* species vary in colour from yellow, orange, red to purple. All the species are native to Chile, Peru and North Brazil, South America, and have recently been introduced into Europe as ornamental plants and hybridized in Europe.

Since no report on the flower pigments in the *Alstroemeria* species and cultivars has been found [1–3] and they have unusual orange or red flower colour, analyses of the flower colour pigments in the several species and cultivars of *Alstroemeria* flowers were undertaken to determine anthocyanin pigments in their flowers.

RESULTS AND DISCUSSION

6-Hydroxycyanidin 3-rutinoside (**1**) was isolated from red flowers of cultivars, 'Red Sunset', 'Red Sunrise' and 'King Cardinal' as a major pigment with 0.1% HCl–MeOH, and purified by TLC (solvents, BAW and Bu-HCl).

This pigment gave a small shift on the addition of $AlCl_3$ showing the presence of free *ortho*-dihydroxyls in the B-ring [1]. Compound 1 showed the λ_{max} at 515 and 282 nm in 0.1% HCl–MeOH, and the value of $E_{440}/E_{vis\ max}$ was 27%. R_f values and spectral data of this anthocyanin and the related pigments are shown in Table 1. On acid hydrolysis, this pigment gave 6-hydroxycyanidin, glucose and rhamnose. Partial acid hydrolysis gave rise to one intermediate, 6-hydroxycyanidin 3-glucoside. Moreover, rutinose was obtained by the H_2O_2 degradation of this pigment. The 100 MHz ^{1}H NMR spectrum [solvent: CD_3OD + DCl (one drop)] of **1** revealed signals at 8.94 at δ (*br s*, H-4), 8.20 (*d*, $J = 2$, 8.5 Hz, H-6′), 8.04 (*d*, $J = 2$ Hz, H-2′), 7.19 (*br s*, H-6 or 8), 7.04 (*d*, $J = 8.5$ Hz, H-5′), 5.39 (*br s*, Glc anomeric H), 4.69 (*br s*, Rha anomeric H), 1.17 (3H, *d*, $J = 6$ Hz, Rha Me), and 10 sugar protons. FAB mass spectrometry established the M_r as 611, *m/z*: $C_{27}H_{31}O_{16}{}^+$. These data indicate that this pigment is 6-hydroxycyanidin 3-rutinoside.

Also, 6-hydroxycyanidin 3-glucoside (**2**) was found in the red flowers of 'Red Sunset' and three other cultivars as a minor component. Compound **2** showed λ_{max} at 512 and 282 nm in 0.1% HCl–MeOH, and the value of $E_{440}/E_{vis\ max}$ was 26%. The 6-hydroxycyanidin skeleton was confirmed by the ^{1}H NMR spectrum.

The aglycone, 6-hydroxycyanidin, obtained from **1** by the complete hydrolysis with 2 N HCl for 1 hr exhibited a bathochromic shift on the addition of $AlCl_3$ (10% in EtOH). This indicates the presence of a dihydroxylic B-ring. It showed λ_{max} at 518 and 282 nm in 0.1% HCl–MeOH, and the value of $E_{440}/E_{vis\ max}$ was 22%. This aglycone showed a low R_f in comparison with those of cyanidin in the Forestal and Formic solvents, and was almost identical with delphinidin (Table 1). These data are identical to those of 6-hydroxycyanidin obtained synthetically by Harborne [1]. The 100 MHz ^{1}H NMR spectrum