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A XANTHONE FROM *CLUSIA INSIGNIS*

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Key Word Index—*Clusia insignis*; Guttiferae; flowers; clusone; xanthone.

Abstract—A new xanthone, clusone, isolated from the fresh flowers of an Amazonian medicinal plant, *Clusia insignis*, has been characterized as 1,3,4,5,6-pentamethoxy 9H-xanthen-9-one by means of spectroscopic evidence. © 1998 Elsevier Science Ltd. All rights reserved

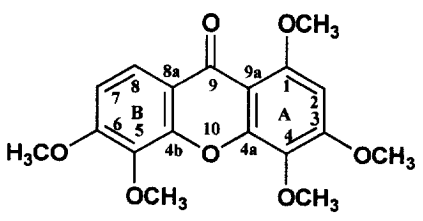
INTRODUCTION

Fresh flowers of the Amazonian medicinal plant, *Clusia insignis*, known locally as "apuí", have been used as a purgative [1]. Despite its long-standing use, there has been little attempt to verify its pharmacological effects. In our continuing search for biologically active substances from tropical plants, the methanol extract of fresh flowers of *C. insignis* was found to exhibit tyrosinase inhibitory activity [2, 3].

RESULTS AND DISCUSSION

The ethanol extract of fresh flowers was suspended in water and extracted with *n*-hexane and ethyl acetate, consecutively. Subsequent bioassays revealed that the activity was retained in the ethyl acetate fraction. During the isolation procedure of tyrosinase inhibitors from this bioactive fraction by repeated chromatography on silica gel using a chloroform and methanol gradient system, a yellow compound was isolated in minute quantities, although this purified compound did not exhibit any notable tyrosinase inhibitory activity.

This yellow compound was designated as "clusone" and the molecular formula, $C_{18}H_{18}O_7$, was established by EI mass spectrometry in conjunction with NMR data. Its structure was established based on spectroscopic data, in particular, the NMR spectra. The IR spectrum showed absorption at 1630 cm^{-1} reminiscent of a xanthone carbonyl group. The UV spectrum was typical of polyoxygenated xanthones [4]. The ^1H NMR spectrum of clusone was relatively simple but showed the presence of five methoxyl groups and three aromatic protons. One aromatic proton gives rise to a singlet at δ 6.48, the other to a doublet at δ 6.88 and δ 8.13 due to *o*-coupling (J = 9.2 Hz). The ^{13}C NMR spectrum of clusone showed 18 signals; *viz.*, one carbonyl group (δ 176.2), 12 aromatic carbons (three *doublets* and nine *singlets*) and five methoxyl groups (δ 56.0, 56.2, 61.0, 61.6 and 61.9). These data were sufficient to consider the possibility of a pentasubstituted xanthone structure for clusone. A doublet at relatively low field at δ 8.13 was characteristic of a C-8 proton, *peri* to the carbonyl group, on an unsubstituted xanthone [5]. The other higher chemical shift at δ 6.88 was then assigned to H-7. In the ^{13}C NMR spectrum, the lower chemical shift value of the methoxyl carbon atoms (δ 61.6 and 61.9) indicated di-*ortho*-substitution [6], suggesting that C-3,4 and C-5,6 were substituted with methoxyl groups. Thus, a singlet proton at δ 6.48 was assigned to H-2. The assignments in the ^{13}C NMR of C-2, C-7 and C-8 were achieved by a CH COSY experiment. The chemical shifts of the remaining xanthone nuclei were assigned with the aid of HMBC correlation (Fig. 1) and by the application of known chemical shift rules [7]. The observation of HMBC cross-peaks between the proton at δ 6.48 (H-2) and C-9a (δ 110.4) provided additional sup-



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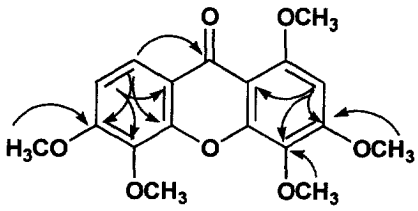

Fig. 1. Long-range correlations in HMBC of clusone (**1**)

port that this proton is located at the 2-position. HMBC cross-peaks of H-2 were also observed between C-3 (δ 158.0) and C-4 (δ 138.2), which showed cross-peaks with methoxyl protons at δ 3.96 and 3.90, respectively. These methoxyl protons were shown to be attached to the methoxyl carbons at δ 56.0 and δ 61.6 by the CH COSY spectra. Thus, methoxyl carbons at δ 61.6 were shown to be attached to C-4 and δ 61.9 to C-5. H-8 showed HMBC correlation to C-4b (δ 134.4) and C-6 (δ 154.0), which showed a cross-peak with the methoxyl protons at δ 3.99 (C-H-correlation with δ_C 56.2). H-7 (δ 6.88) showed HMBC correlations to C-5 (δ 133.6), C-8a (δ 117.5) and carbonyl carbon. The final structure of clusone was established as 1,3,4,5,6-pentamethoxy 9H-xanthen-9-one (**1**). Although xanthones are a common constituent from the Guttiferae [8], this xanthone appears to be the first report from a *Clusia* species.

Clusone was isolated from the tyrosinase inhibitory fraction but it did not show any activity at 185 μg/ml [2, 3]. It should be noted that since this xanthone is sparingly soluble in water, higher concentrations could not be tested. However, it is evident that clusone should not have high tyrosinase inhibition activity judging from its chemical structure. In addition, the compound did not exhibit any other biological activities, such as antimicrobial [9], insect [10] and plant growth inhibitory activities [11].

EXPERIMENTAL

General

General procedures are the same as those used in previous work [2, 3]. ^{1}H and ^{13}C NMR were taken in $CDCl_3$.

Plant material

Fresh flowers of *C. insignis* were purchased at open market places in Belém, Brazil, and immediately immersed in EtOH. The species was identified by Dr J. M. Pines, a botanist at Museu Goeldi, Belém.

Isolation and identification

Fresh flowers (500 g) were extracted with EtOH (×3) at ambient temp. After evapn of solvent under red. pres., a brown residue (8.8 g) was obtained. This was suspended in H_2O and the suspension successively partitioned with *n*-hexane and EtOAc. Subsequent bioassays showed that the EtOAc fr. had retained the activity. The EtOAc fr. (1.1 g) was chromatographed on silica gel repeatedly using a $CHCl_3$ and MeOH gradient system. This yielded 69 mg of clusone.

1,3,4,5,6-*Pentamethoxy-9H-xanthen-9-one* (**1**)

Pale yellow needles, mp 225–230°. Positive EI-MS *m/z* (rel. int.): 346 $[M]^+$ (12), 345 $[M-H]^+$ (46), 330 (100), 315 (26), 165 (17). UV λ^{MeOH} nm (log ϵ): 225 (4.35), 269 (4.87), 321 (3.82), 375 (3.82). IR γ^{KBr} cm^{-1}: 1630, 1620, 1600, 1275. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.90 (3H, *s*, 4-OCH_3), 3.96 (3H, *s*, 3-OCH_3), 3.99 (3H, *s*, 6-OCH_3), 4.02 and 4.03 (3H, *s*, 1 or 5-OCH_3), 6.48 (1H, *s*, 2-H), 6.88 (1H, *d*, J = 9.2, 7-H), 8.13 (1H, *d*, J = 9.2, 8-H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 56.0 (C3-OCH_3), 56.2 (C6-OCH_3), 61.0 (C1-OCH_3), 61.6 (C4-OCH_3), 61.9 (C5-OCH_3), 93.8 (C-2), 107.0 (C-7), 110.4 (C-9a), 117.5 (C-8a), 123.1 (C-8), 133.6 (C-5), 134.4 (C-4b), 138.2 (C-4), 139.4 (C-4a), 154.0 (C-6), 154.7 (C-1), 158.0 (C-3), 176.2 (C-9).

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