CONSTITUENTS OF *Ipomoea horrida* HUBER EX DUCKE: SPECTROSCOPIC IDENTIFICATION OF THE FLAVONOIDS

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ABSTRACT

As part of a systematic investigation of the *Ipomoea* species of Northeastern Brazil, a mixture of 7,4'-di-O-methylkaempferol and 7,3',4'-tri-O-methylquercetin were identified in extracts obtained from the aerial parts of *Ipomoea horrida*.

Uniterms: *Ipomoea horrida*; Convolvulaceae; Flavonoids.

INTRODUCTION

Various species of *Ipomoea* (Convolvulaceae) are widley used in popular medicine[1]. Because of the presence of indole alkaloids and of antitumor activity[2], several *Ipomoea* species have also been investigated pharmacologically[3]. Our interest has been to conduct a systematic botanical survey and microbiological screening of the *Ipomoea* species growing in the various phytogeographic regions of Northeastern Brazil, followed by a detailed chemical investigation. Thus, the $CHCl_3$ extract of the aerial parts of *I. horrida* Huber ex Ducke yielded a slightly greenish yellow substance (substance F) which was practically insoluble in MeOH, $CHCl_3$ and other common organic solvents. In this paper, we describe the identification of F as a mixture of 7,4'-di-O-methylkaempferol and 7,3',4'-tri-O-methylquercetin by the use of MS and ^{13}C NMR spectroscopy of the natural products and the methylated derivatives. The ^{13}C NMR spectrum of 7,3',4'-tri-O-methylquercetin as well as its methylated derivative are being reported here for the first time.

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EXPERIMENTAL

Plant material. The aerial parts of *Ipomoea horrida* were collected near Campina Grande, State of Paraíba in August 1995. A voucher specimen (Agra 748) is deposited at the Lauro Pires Xavier Herbarium (JPB), João Pessoa, PB, Brazil.

Extraction and isolation of the constituents. The air-dried and powdered plant material (500 g) was percolated with 95% EtOH a room temperature and the EtOH extract was subsequently concentrated *in vacuo*. The residue was suspended in 60% MeOH and the resulting mixture was extracted with hexane followed by $CHCl_3$. The $CHCl_3$ extract, after usual treatment was chromatographed over a Silica gel column eluting with $CHCl_3$ containing increasing amounts of MeOH. The residue of fractions 48-52 was found to be a greenish-yellow solid (substance F, 0.21 g) which failed to crystallize. The substance F was found to be insoluble in acetone, $CHCl_3$ and MeOH. However, it was soluble in dilute NaOH solution.

Methylation. The substance F (0.05 g) was treated with excess CH_2N_2. The product, after initial purification, followed by GC-MS analysis, gave only one peak with retention time of 32.5 min and a molecular ion M^+ at m/z 328. This is referred to as F-Me-A. The methylation of substance F was repeated with the product left standing for several hours. The resulting material also failed to crystallize. However, upon GC-MS analysis, the methylated product showed the presence of two peaks with retention times of 36.3 min and 41.8 min, corresponding to the molecular ions M^+ at m/z 342 and 372, respectively. These are referred to as F-Me-B and F-Me-C.

RESULTS AND DISCUSSION

We have earlier demonstrated the versatility of the use of ^{13}C NMR spectroscopy as well as GC-MS in the identification of relatively common plant constituents which resist usual separation methods[4-6]. The MS of F-Me-A, in addition to the M^+ at m/z 328, gave significant fragments at m/z 327 (95%, M-1), 313 (19%, M-Me), 285 (40%, M^+-Me-CO), 167 and 135. The peaks at m/z 167 and 135 are the $[A_1+H)^+$ ion produced by pathway I and B_2^+ by pathway II from the flavonols, respectively, by the usual retro Diels Alder fragmentation of the heterocyclic ring[2]. The intense M-1 peak accompanied by $[M-CH_3]^+$ and $[M-CH_3-CO]^+$ confirm the flavonol structure.

The peak at m/z 135 (B_2^+) is only possible if the ring B has a OCH_3 substituent and the peak at m/z 167 $[A_1+H]^+$ is indicative of the presence of a OH and a OCH_3 at ring A.

The MS of F-Me-B, in addition to the very strong peak of M^+ at m/z 342 (14 mass units higher than that of F-Me-A) also shows the base peak at m/z 341 (M-1) and significant peaks at 323 $[M-CO-H]^+$ and 135 (B_2^+). The last two peaks are also present in the MS of F-Me-A. On the basis of these observations, it is reasonable to assume that F-Me-B is the complete and F-Me-A is a partial methylated product of one of the components (F-1) of substance F. Also, the ring B of both these methylated products contain only one OCH_3 substituent (m/z 135).

The MS of substance F clearly shows the lack of homogeneity of the material. However, the strongest peak appears at m/z 314 and another very strong peak at 344. It seems reasonable that the peak in the spectrum at m/z 314 is due to the M^+ of the component F-1 of which F-Me-A (M^+ at m/z 328) and F-Me-B (M^+ at m/z 342) are, respectively, the mono- and di-methylated products. This is further supported by the fact that the peaks at m/z 167 and 135 arising from ring B are also present in the MS of substance F. This definitely confirms that the ring B of the component F-1 contains one OCH_3 group which did not change upon methylation (vide infra), and the ring A contains in addition to a OCH_3, a OH group which is difficul to methylate such as the C-5-OH, *peri* to C-4-C=O group. Therefore, the initial treatment of CH_2N_2 resulted in the formation of a partially methylated product (F-Me-A, M^+ at 328) in which only the C-3-OH group must have been methylated.

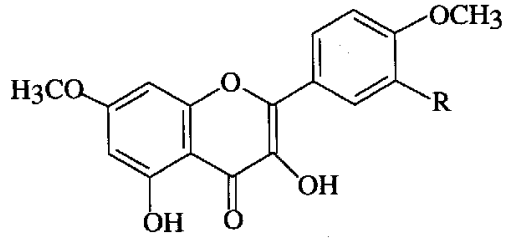
The above facts certainly indicate that the second component (F-2) of the substance F shows the M^+ at m/z 344; 30 mass units higher than that of F-1. The compound F-2 upon methylation gave F-Me-C (M^+ at m/z 372). The small but significant peak at m/z 165 in the MS of substance F, therefore, appears to be the B_2^+ peak of F-2 which certainly has two OCH_3 groups at ring B. The absence of any other significant peak in the MS of substance F suggests that rings A of both F-1 and F-2 are identical with a OCH_3 and a 5-OH group and the ring B of F-1 contains only one OCH_3 group whereas that of F-2, two OCH_3 groups.

The proton decoupled ^{13}C NMR spectrum of substance F (Table 1) showed 20 signals of unusually varying intensities indicating the lack of homogeneity of the sample. Only three of these signal appear at 55.9, 55.6 and 55.3 ppm which are obviously due to OCH_3 groups and the rest of the signals appear downfield from 90.0 ppm, typical of flavonols. The intense singlets at 176.0, 146.5 and 136.5 ppm are diagnostic of C-4-C=O, C-2 and C-3, respectively, of flavonols and the chemical shift of the C=O (176.0) confirms the presence of a 5-OH group[7]. The unusual high intensity of these singlets

clearly suggests that they represent 2 carbons each. The intense doublets at 97.3 and 91.9 ppm are typical of C-6 and C-8 of 5,7-dioxygenated flavonols[7]. All these signals are indicative of the presence of two compounds in F, both having the identical substitution pattern at ring A, differing in ring B. The very intense doublets at 129.3 and 113.9 ppm are characteristic of C-2'/C-6' and C-3'/C-5'of a *para* disubstitued ring B of flavonols[7]. This, along with MS evidence confirm the presence of a OCH_3 group at C-4' at ring B of compound F-1.

The MS and ^{13}C NMR spectra of F, therefore, reveal that one of the constituents (F-1) must be 7,4'-dimethoxykaempferol (Table 1), a compound which has been isolated previously from other sources[8] although never before from an *Ipomoea* species.

The low intensity doublets at 111.1, 111.4 and 121.5 ppm in the spectrum of F are indicative of the presence of a compound (F-2) having a 3',4'-dioxygenated ring B and substitution at ring A identical to that of F-1. Therefore, the MS and ^{13}C NMR spectra confirm that F-2, the second constituent of F must be 7,3',4'-trimethoxyquercetin. Although this compound was encountered in nature earlier[9, 10], this is the first report of its presence in *Ipomoea*. Also, to our knowledge, the ^{13}C NMR data of this compound is being reported here for the first time.



(F-1) R=H
(F-2) R=OCH_3

RESUMO

Como parte de uma investigação sistemática das espécies de *Ipomoea* do Nordeste do Brasil, foi identificado uma mistura de 7,4'-di-O-metilkaempferol e 7,3',4'-tri-O-metilquercetina isoladas das partes aéreas de *Ipomoea horrida*.

UNITERMOS: *Ipomoea horrida*, Convolvulaceae, Flavonóides

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Table 1. ^{13}C NMR spectral data (in ppm) of flavonols from *Ipomoea horrida* in DMSO-d_6.

Carbons	2	3	4	5	6	7	8	9	10	1'	2'	3'	4'	5'	6'	OCH_3
7,4'-di-O-methylkaempferol (F-1)	146.4	136.5	176.0	156.1	97.3	164.9	91.9	160.5	104.1	123.3	129.3	113.9	160.5	113.9	129.3	55.9
																55.6
Substance F	146.4	136.5	176.0	156.1	97.3	164.9	91.9	160.5	104.1	123.3	111.1	148.4	151.0	111.4	121.5	55.9
											129.3	113.9	160.5	113.9	129.3	55.6
																55.3
7,3',4'-tri-O-methylquercetin (F-2)	146.4	136.5	176.0	156.1	97.3	164.9	91.9	160.5	104.1	123.3	111.1	148.4	151.0	111.4	121.5	55.9
																55.6
																55.3
Methylated substance F	151.9	140.6	173.4	158.3	95.3	163.5	92.1	160.5	108.9	122.8	129.3	113.5	160.7	113.5	129.3	59.4
											110.5	148.3	150.5	110.9	121.2	56.0
																55.6
																55.0