DITERPENES AND ALKALOIDS FROM BRAZILIAN *XYLOPIA* SPECIES

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The isolation of tetracyclic diterpenes, aporphine and benzyltetrahydroisoquinoline alkaloids from the wood of *Xylopia amazonica* and *X. aromatica* is described.

Keywords: diterpenes; alkaloids; *Xylopia*; Annonaceae.

INTRODUCTION

The genus *Xylopia* belonging to the Annonaceae family has ca 160 species[1] and is widely distributed in the tropical and subtropical regions[2]. The family located in the Magnoliales, a primitive order of plants, is known to produce benzyltetrahydroisoquinoline alkaloid derivatives. The African species, despite of thorough investigation of the studied plants, have failed to produce the typical alkaloids from Annonaceae yielding diterpenes as the major secondary metabolites[3].

We are studying the wood of *X. amazonica* and *X. aromatica* in continuation to a phytochemical investigation of Brazilian *Xylopia* species. From the fruits of these species, *X. emarginata* and *X. brasiliensis* we have previously isolated diterpenes[4,5,6]. The bark of the last one gave aporphine alkaloids[7].

In the present paper we report the isolation of diterpenes and alkaloids from the wood bark of *X. amazonica* and alkaloids from the bark of *X. aromatica*.

RESULTS AND DISCUSSION

Xylopia amazonica R.E. Fries is a large tree that grows in the Amazonia region of Brazil. From the hexane extract of the wood bark we have isolated three kaurene (**1-3**) and one beyerene (**4**) diterpenes besides β-sitosterol. The hexane extract of the wood gave the same diterpene compounds found in the wood bark, and the dichloromethane extract afforded four alkaloids: one oxoaporphine (**5**), two aporphines (**6,7**) and one noraporphine (**8**).

Kaurene diterpenes are typical of *Xylopia* species, in spite of that, this is the first time that 4-epi-kaurenoic acid (**3**) is isolated from this genus. In fact, we did not find any report on the isolation of **3** from plants.

Xylopia aromatica Lam. (Mart.) is a tree of common occurrence throughout the Brazilian territory. Ethanol and dichloromethane extracts of the bark from three specimens collected in different regions of Brazil: Minas Gerais (Southeast), Bahia (Northeast) and Mato Grosso do Sul (Middle West) during the 1990 summer, were examined by TLC. The behaviour of the dichloromethane extracts in the chromatoplates were almost identical. The same occurred with the ethanol extracts. These observations allowed to suggest that the chemical constitution of the bark of *X. aromatica* does not have an observable change, with the change of geographical localization of the plant.

The bark collected in Minas Gerais was extracted with dichloromethane and ethanol. ^{1}H NMR spectrum of the first extract showed diterpenes as the major constituents of this extract. The ethanol extract was submitted to an acidic extraction to give an alkaloid mixture. From the less polar fractions of a silica column of this mixture 10 alkaloids were isolated: six aporphines (**6,7,9,10,11,13**), two noraporphines (**12,14**), one dehydroaporphine (**15**) and one benzyltetrahydroisoquinoline (**16**).

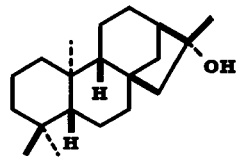
The identification of the known alkaloids and diterpenes was based on spectroscopic data from the literature as indicated in the experimental. The structural determination of 4-epi-kaurenoic acid was achieved by the analysis of the IR, EM, ^{1}H and ^{13}C NMR spectra, comparison of the ^{13}C NMR spectrum of kaurenoic acid (**2**) with that of **3** shows the only difference between them is the C-4 stereochemistry.

EXPERIMENTAL

Xylopia amazonica R.E. Fries was collected near Costa Marques, State of Rondonia, in the Amazonian forest. A voucher is deposited in the herbarium of Instituto Nacional de Pesquisas da Amazonia (INPA). Dried and powdered wood bark (3.0 kg) of *X. amazonica* was extracted at room temp. with hexane for three days. The hexane extract (10 g) was recrystallized from CH_2Cl_2 to give **1** (250 mg). Physical and spectral data of **1** agree with the literature[5]. The filtrate was submitted to silica gel column chromatography eluted with hexane, CH_2Cl_2 and EtOAc in mixtures of increasing polarity. After CH_2N_2 methylation, some fractions have been purified by prep. TLC on silica gel plates impregnated with 5% $AgNO_3$ to give **2** (40 mg) and **3** (60 mg) as methyl esters and **4** (30 mg). The identification of **2** and **4** was based on spectral data from references 6 and 8 respectively.

Methylkaur-16-en-18-oate (**3**): mp 98-100° (hexane). IR: KBr (cm^{-1}) 3069, 2947, 2864, 1717, 1656, 1479, 1453, 945, 907, 809. ^{1}HNMR (80 MHz, $CDCl_3$, ppm) 4.77-4.74 (m, 2 H), 3.64 (s, 3 H); 2.60 (m, 1 H), 1.04 (s, 3 H); 1.16 (s, 3 H); ^{13}C NMR (20 MHz, $CDCl_3$, ppm) 40.5 (C-1), 17.9 (C-2), 36.7 (C-3), 47.6 (C-4), 50.3 (C-5), 23.0 (C-6), 38.5 (C-7), 44.1 (C-8), 55.8 (C-9), 39.6 (C-10), 17.7 (C-11), 33.0 (C-12), 43.8 (C-13), 39.5 (C-14), 48.9 (C-15), 155.4 (C-16), 102.8 (C-17), 179.2 (C-18), 16.2 (C-19), 17.7 (C-20), 51.6 (OCH_3). EM: m/z (rel %) 316 (22), 257 (46), 241 (75), 215 (4), 213 (43), 121 (86), 109 (50), 93 (56), 91 (100).

The wood (6.0 kg) of *X. amazonica* was submitted to hexane and CH_2Cl_2 extractions under the same conditions as the wood bark. A TLC of the hexane extract (13.0 g) revealed the same chemical constituents as the hexane extract of the wood bark. The CH_2Cl_2 extract (17.0 g) gave a



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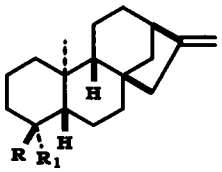
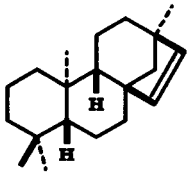


2. R=CH_3; R_1=CO_2H
3. R=CO_2H; R_1=CH_3



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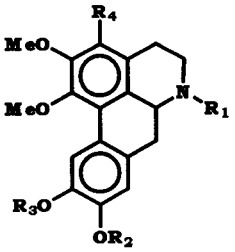
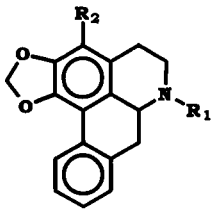
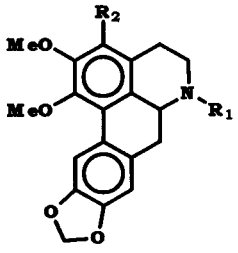


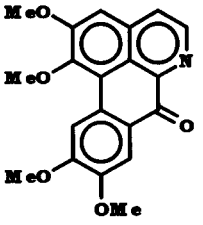
6. R_1=R_2=R_3=Me; R_4=H
7. R_1=R_2=Me; R_3=R_4=H
8. R_1=R_2=R_4=H; R_3=Me
9. R_1=R_2=R_3=Me; R_4=OMe



10. R_1=Me; R_2=H
11. R_1=Me; R_2=OMe
12. R_1=H; R_2=OMe



13. R_1=Me; R_2=H
14. R_1=H; R_2=OMe



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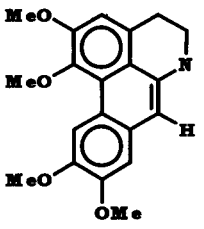

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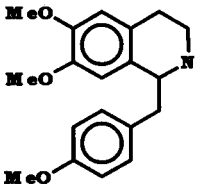

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positive Dragendorff test, thus it was submitted to an alkaloid extraction with AcOH 10% aqueous solution. The acidic solution was extracted with $CHCl_3$. After alkalinization, the aqueous phase was extracted with $CHCl_3$. The $CHCl_3$ extract (250 mg) was chromatographed on a silica gel column eluted with $CHCl_3$-MeOH (97:3) to give **5** (50 mg), **6** (20 mg), **7** (20 mg) and **8** (30 mg).

Samples of the bark of *Xylopia aromatica* Lam (Mart). were collected during February of 1990 in three regions: Coqueiral, Minas Gerais (Southeast); Salvador, Bahia (Northeast) and Campo Grande, Mato Grosso do Sul (Middle West) and extracted with CH_2Cl_2 and EtOH successively. The extracts were then submitted to several TLC on silica gel eluted with: hexane-EtOAc (8:2); CH_2Cl_2-MeOH (95:5) and $CHCl_3$-MeOH (9:1). The chromatoplates were revealed with iodine vapour, cerium (IV) sulphate, Dragendorff and iodoplatinate.

Dried and powdered bark (1.5 kg) of *X. aromatica* from Coqueiral, M. G. was extracted with CH_2Cl_2 and EtOH under the same conditions as the wood bark of *X. amazonica*. The EtOH extract (70 g) gave a positive Dragendorff test, thus it has been submitted to an alkaloid extraction with AcOH 10% aqueous solution. The acidic solution was extracted with EtOAc. After alkalinization the aqueous solution was extracted with EtOAc to give the alkaloid extract (4 g). The extract was chromatographed on a silica gel column eluted with a gradient mixture of CH_2Cl_2-MeOH. The less polar fractions were then submitted to an alumina column eluted with hexane-EtOAc-MeOH gradients. Prep. TLC on alumina of the first fraction of the column eluted with hexane-AcOEt (7:3) afforded **6** (100 mg), **9** (10 mg), **10** (4 mg), **11** (4 mg).

Fractions 2-6 submitted to a flash alumina column (hexane-EtOAc 7:3) and then to prep. TLC on alumina eluted with hexane-EtOAc (7:3) gave **6** (54 mg), **9** (6 mg), **12** (15 mg), **13** (2 mg), **15** (7 mg), **16** (7 mg) and hexane-EtOAc-MeOH (6:4:0.2) gave **14** (17 mg). The more polar fractions after a prep. TLC on silica gel eluted with CH_2Cl_2-MeOH (95:5) gave **7** (42 mg).

The identification of the alkaloids was based on physical and spectral data using the following references: **5**, oxoglaucine[9a]; **6**, glaucine[9a,b]; **7**, lirioferine[9b,d]; **8**, laurotetanine[9a]; **9**, purpureine[9a,d]; **10**, roemerine[9a]; **11**, stephalagine[9a]; **12**, norstephalagine[9b,c]; **13**, nantenine[9a]; **14**, norphoebine[9d]; **15**, dehidroglaucine[9c,d]; **16**, O-methylarmepavine[10].

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