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ALKALOIDS OF SOME MEXICAN *ZANTHOXYLUM* SPECIES*

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Key Word Index—*Zanthoxylum arborescens*; *Z. limoncillo*; *Z. caribaeum*; *Z. fagara*; Rutaceae; quinazolone alkaloids; skimmianine; scopeletin; alkaloid synthesis; ^{13}C NMR.

Abstract—Two new 4-quinazolone alkaloids have been isolated from seed husks of *Zanthoxylum arborescens*. Based on their spectroscopic properties they have been assigned structures, 1-methyl-3-(2′-phenylethyl)-1H,3H-quinazoline-2,4-dione and 1-methyl-3-[2′-(4″-methoxyphenyl)ethyl]-1H,3H-quinazoline-2,4-dione. These structural assignments have been confirmed by synthesis. Skimmianine has been obtained from leaf extracts of *Z. dimoncillo* and *Z. caribaeum* while skimmianine and scopeletin have been isolated from leaf extracts of *Z. fagara*.

INTRODUCTION

Much recent chemotaxonomic effort in the family Rutaceae has focused on the botanical relationship of two related genera, *Zanthoxylum* and *Fagara*. The exact botanical relationship of these two genera is not clear. The classical treatment [1] maintains two genera but some authorities [2] believe that both genera should be combined.

Some previous chemical work in this area has explored the possibility of using chemotaxonomy as an aid in establishing the relationship of *Zanthoxylum* to *Fagara* [3]. Much of the previous chemical work on these genera has been carried out on old world species. This paper described chemical studies on several Mexican *Zanthoxylum* species [4]. *Z. arborescens* (Rose) is indigenous to the southern tip of Baja, California. Work-up of the foliage extracts failed to yield any tractable materials. However, the seed husk extracts, after chromatography, gave two new closely related alkaloids.

RESULTS AND DISCUSSION

The major alkaloid, mp 100–102°, was blue fluorescing on TLC and gave a negative ferric cholride test. Its IR spectrum showed three intense bands in the carbonyl–aromatic region at 1702, 1655 and 1620 cm^{-1}. The ^1H NMR showed two two-proton multiplets at 2.93 and 4.40 ppm, an *O*-methyl or *N*-methyl three-proton singlet at 3.55 ppm and a complex pattern in the aromatic region representing nine protons. The only interpretable features in the aromatic region was a one-proton downfield quartet ($J = 7,1$ Hz) centered at 8.12 ppm and a one-proton triplet at 7.8 ppm ($J = 7$ Hz) with each signal further split ($J = 1$ Hz). This aromatic pattern suggested the presence of four adjacent aromatic protons on an *o*-substituted benzene ring. The extreme downfield aromatic quartet at 8.12 ppm indicated the presence of an adjacent carbonyl group. These NMR data with consideration of the MS suggested the presence of an *N*-methylanthranilic acid system.

The MS fragmentation supported the presence of the *N*-methylanthranilic acid system. These include the fragments at *m/e* 176, 133 and 105 which are assigned structures indicated in Scheme 1. The relationship between these fragments and the M^+ was supported by the presence of appropriate metastable peaks.

Consideration of the ^1H and ^{13}C NMR spectra of a number of model anthranilic acid derivatives supported the presence of the *N*-methylanthranilic acid system (Table 1). Moreover, the position of the carbonyl resonance in the ^{13}C NMR at 161.4 ppm indicated that it was an amide carbonyl [5]. The same spectrum indicated that the methyl singlet observed in the ^1H NMR must be due to an *N*-methyl group rather than *O*-methyl since methoxy resonances fall in the narrow range of 51–65 ppm and this region was clear of signals in the ^{13}C NMR.

The two two-proton multiplets (2.93–4.40 ppm) in the ^1H NMR were consistent with the presence of a 2-phenylethyl system. A series of MS fragments at *m/e* 105, 104 and 91 also supported the presence of the 2-phenylethyl group. A high resolution MS indicated the molecular formula of $C_{17}H_{16}N_2O_2$. With this empirical formula, the 2-phenylethyl and *N*-methylanthranilic amide system could be assembled in two different ways leading to structures **1** or **2**.

The MS of **1** and **2** would not be expected to be different enough to distinguish between these two structures. The ^{13}C NMR of the alkaloid did not possess any signals in the 51–75 ppm region suggesting

*Part XIII in the series "Chemotaxonomy of the Rutaceae". For Part XII see Dreyer, D. L., Rigod, J. F., Basa, S. C., Mahanty, P. and Das D. P., (1980) *Tetrahedron* (in press).

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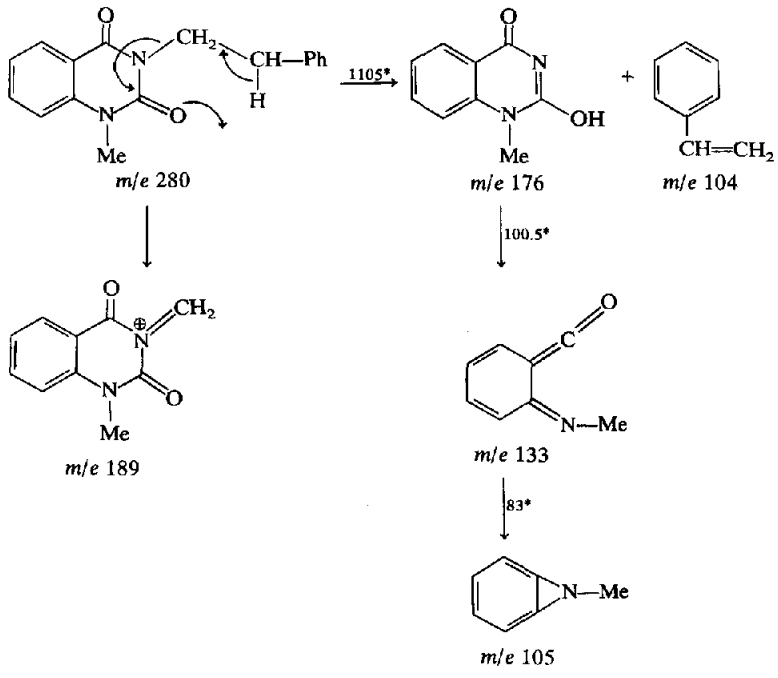


Scheme 1. MS fragmentation of the major *Zanthoxylum* alkaloid.

the absence of carbons singly bonded to oxygen. This fact would support structure **2**. The lack of signals in this region also exclude the presence of methoxy groups and hence the three-proton singlet at 3.55 ppm in the ^{1}H. NMR must be due to an *N*-methyl group. Structure **2** contains a urea carbonyl group. Carbon signals for urea carbonyls fall in the range of 146–165 ppm [6] well into the vinyl–aromatic region. This feature of the ^{13}C NMR cannot then be used to uniquely distinguish structure **1** from **2**.

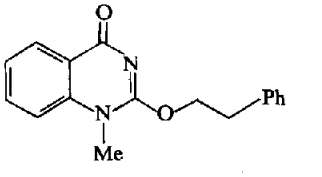
The ^{13}C NMR assignments were made by comparison of the chemical shifts with those of simpler model compounds. Thus, by using the literature values for methyl salicylate and methyl anthraniliate [7] assignments could be made to 1,2-dimethyl-4-quinolone, 1-methyl-2,4-quinazolsione and, in turn, to the heterocycle ring of the alkaloid (Table 1).

The assignments to the methylene groups were less straightforward. The assignments to the two methylene carbons made by comparison with the corresponding carbon resonances in 2-phenylethanol (C-1 at 63.2 and C-2 at 39.1 ppm) where there was no ambiguity. In 2-phenylethylamine the signal assigned to C-1 occurred at 43.7 and to C-2 at 40.2 ppm; in *N*-(2-phenylethyl)-benzamide C-1 occurred at 36.4 and C-2 at 41.9 ppm and in *N*-(2-phenylethyl)phthimide at 34.3 and 39.0 ppm, respectively.

Since the evidence available to distinguish between structures **1** and **2** was largely permissive, a decision between the two possibilities was sought through synthesis. The alkaloid was synthesized from *N*-methylisatoic anhydride (**3**) and 2-phenylethyl amine in a straightforward fashion (Scheme 2).

The intermediate (**5**) could not be isolated from the reaction mixture but instead only the desired ring closed material (**2**) was obtained. The synthetic product, 1-methyl-3-(2′-phenylethyl)-1H,3H-quinazoline-2,4-dione (**2**) was identical with a natural sample (mmp and spectroscopic criteria).

A methoxy analogue of **2** was recovered from the



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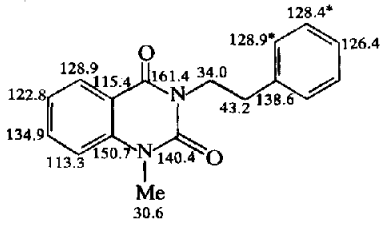

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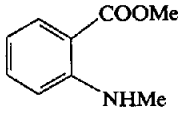
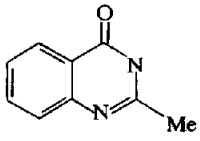
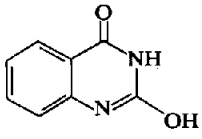
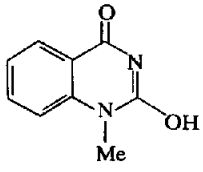
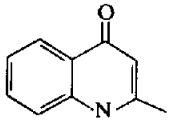
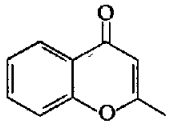
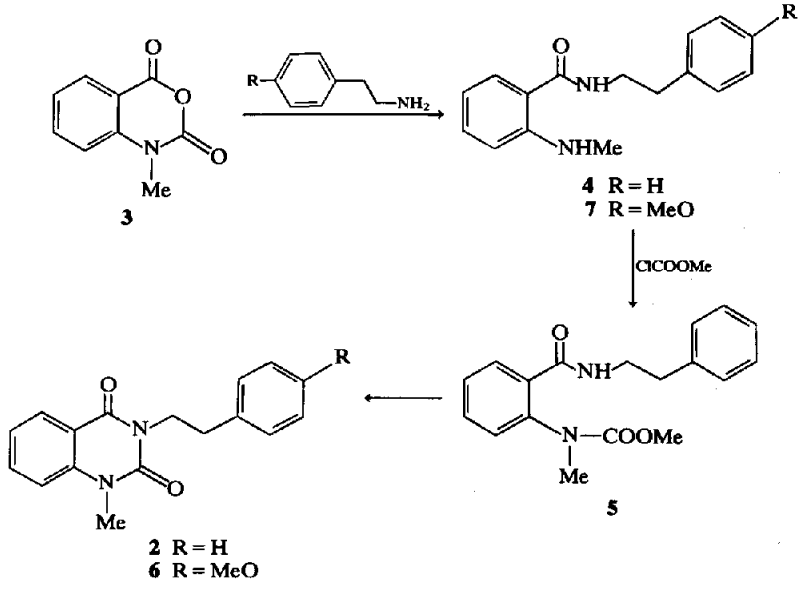
mother liquors of the initial isolation procedure. Its IR spectrum showed intense bands at 1700, 1660 and 1620 cm^{-1}. The high resolution MS indicated a molecular formula of $C_{18}H_{18}N_2O_3$. The ^{1}H NMR spectrum had many similarities to that of the major alkaloid. The aromatic region showed an A_2B_2 system (6.90 and 7.20 ppm) as well as those signals associated with the *N*-methylanthranilic acid system. Two three-proton singlets at 3.58 and 3.77 ppm suggest the presence of both *N*-methyl and methoxy groups. The multiplets assigned to the two adjacent methylenes had *ca* the same chemical shifts as in **2** (2.87 and 4.44 ppm). The MS was similar to that of **2** except for those fragments containing the *p*-methoxyphenylethyl group.

These data indicated structure **6** for the second metabolite. This assignment was confirmed by synthesis along the same route as that employed for the major alkaloid (Scheme 2).

Quinazolone alkaloids have been previously isolated from rutaceous plants [8–14] and metabolites based on

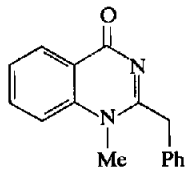
Table 1. ^{13}C NMR data of anthranilic acid derived model substances

	Carbon								
	2	4	5	6	7	8	4a	8a	Other assignments
		168.9	131.6	114.3	134.6	110.7	109.9	152.0	N-Me 29.4 O-Me 51.2
	155.2	162.2	126.9	126.1	134.4	126.1	121.2	149.5	C-Me 21.7
	141.1	163.0	127.0	115.5	134.9	114.5	122.4	150.5	
	141.6	161.7	127.2	122.3	135.1	114.5	115.5	150.2	N-Me 29.4
	142.9	177.2	126.7	123.4	132.5	116.8	—	152.5	C-Me 22.0 N-Me 34.6 C-3 111.6
	165.2	176.7	124.4*	123.9	132.4	116.9	122.5	155.3	C-Me 19.5 C-3 109.5



Scheme 2. Synthetic route to quinazoline-2,4-diones.

N-methylanthranilic acid, 2-phenylethyl amine and tyramine are also well represented in the same family [15]. The exact combination in nature of these fragments in the fashion of structures **2** and **6** has not, however, been previously recorded. Nevertheless **4** or an oxidized equivalent is a reasonable biosynthetic intermediate to both **2** and arborine (**8**), which is found in *Glycosmis pentaphylla* (Rutaceae) along with glycosmicine, 1-methyl-1H,3H-quinazoline-2,4-dione (**9**) [13, 14].



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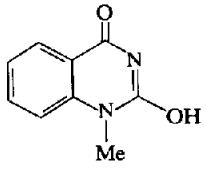

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Work-up of foliage extracts of *Z. limoncillo* and *Z. caribaeum* Lam. by chromatography yielded skimmiamine in each case. The foliage extracts of the widely distributed *Z. fagara* (L.) Sarg. yielded both skimmiamine and scopeletin after chromatography. These extractives are unexceptional for rutaceous species and skimmiamine is certainly the most characteristic alkaloid of this plant family [16].

The alkaloids 5-methoxycanthin-6-one, *N*-methyl isocorydine and berberine have previously been reported [17, 18] from the bark of *Z. caribaeum* while lauriforine and magnoflorine have been reported from that of *Z. fagara* [19].

EXPERIMENTAL

Isolation from Z. arborescens. *Z. arborescens* was collected at two sites in Baja, California, Mexico; 10.5 miles S. of San Pedro on Mexican highway #1 and 6 miles from the junction with Mexican highway #1 on the road to Todos Santos. Ground seed husks were extracted with petrol in a Soxhlet extractor. Solvent was removed from the extracts and the residue chromatographed on Si gel. Fractions eluted with petrol–C_6H_6 mixtures were worked up to give **2**, mp 100–102°(EtOAc–petrol); λ_{max}^{EtOH} nm: 221 (61 000), 240, 310 (4100); no shift with added base; negative $FeCl_3$ test; MS *m/e* (rel. int.): 280 (67) M^+, 189 (28), 177 (35), 176 (100), 134 (27), 133 (28), 132 (15), 105 (40), 104 (77), 91 (15), 78 (17), 77 (27), 56 (15); M^+ 280.1226 (calc. for $C_{17}H_{16}N_2O_2$, 280.1211. Rechromatography of the mother liquors and work-up of fractions showing fluorescence on TLC gave **6**, mp 133–134° (EtOAc–petrol); λ_{max}^{EtOH} nm: 222 (61 000), 242, 276 (3200), 283 (3200), 310 (4100); MS *m/e* (rel. int.): 311 (10), 310 (45) M^+, 155 (11), 135 (100), 134 (52), 121 (75), 119 (54), 58 (20), 57 (16); M^+ 310.1333 (calc. for $C_{18}H_{18}N_2O_3$, 310.1317).

2-*Methylamino*-N-(2′-*phenylethyl*)*benzamide*(**4**) [20]. To 8.2 g *N*-methylisatoic anhydride (**3**) [15] was added 6 g 2-phenylethyl amine in dioxan. The mixture became warm and gas was evolved. After gas evolution ceased the mixture was warmed at 100° for 10 min. Excess H_2O was added to the cooled reaction mixture and a heavy oil separated which crystallized after scratching and cooling. The product was collected by filtration and washed well with aq. carbonate and then H_2O, mp 77–78° (recrystallized twice MeOH). (Found: C, 75.5; H, 7.15. $C_{16}H_{18}N_2O$ requires: C, 75.56; H, 7.13%).

1-*Methyl*-3-(2′-*phenylethyl*)-1H,3H-*quinazoline*-2,4-*dione* (**2**). To a soln of 26.4 g of the amide (**4**) in dioxan was added an aq. soln of Na_2CO_3 followed by 10 ml methyl chloroformate. The soln was stirred at room temp. for 20 min. A large excess of H_2O was added and the product (**2**) crystallized with scratching. The product was collected by filtration, washed with dil HCl and 5% aq Na_2CO_3; mp 101–102° (recrystallized twice MeOH). The NMR and IR spectra were identical with those of a natural sample. (Found: C, 73.1; H, 5.95. $C_{17}H_{16}N_2O_2$ requires: C, 72.84; H, 5.75%).

2-*Methylamino*-N-[2′-(4″-*methoxyphenyl*)*ethyl*]*benzamide* (**7**). To 14 g *N*-methylisatoic anhydride (**3**) [21] in dioxan was added 12 g *p*-methoxyphenylethylamine. The mixture was warmed at 100° for 20 min, cooled and diluted with H_2O. The product crystallized and was collected by filtration, mp 97–98° (MeOH). (Found: C, 71.9; H, 7.17. $C_{17}H_{20}N_2O_2$ requires: C, 71.81; H, 7.09%).

2-*Methylamino*-N-[2′-(4″-*methoxyphenyl*)*ethyl*]*benzamide line*-2,4-*dione* (**6**). To a soln of 10.5 g of the benzamide (**7**) in 2-methoxyethanol and dil aq. Na_2CO_3 was added 4 ml methyl chloroformate. The mixture was stirred for 20 min at room temp. and then diluted with a large excess of H_2O. The product did not crystallize readily. The mixture was extracted with $CHCl_3$, dried and filtered through a short column of Al_2O_3. Solvent was removed from the filtrates and the oily residue crystallized in relatively poor yield, mp 135–136° (MeOH–H_2O or EtOAc–hexane) mmp 133–135°. The IR and NMR spectra were identical with those of a natural sample. (Found: C, 69.7; H, 5.89. $C_{18}H_{18}N_2O_3$ requires: C, 69.66; H, 5.85%).

Isolation from Z. limoncillo. Plant material was collected in southern Mexico, 11 miles NE of La Trinitaria along the road to Lago de Montebello, Chiapas. Me_2CO extracts of the dried foliage gave, after chromatography on Si gel, skimmainene. The IR spectrum was identical with that of an authentic sample.

Isolation from Z. caribaeum. Plant material was collected in a dry arroyo 2.5 miles from the junction of Mexican highway #15 on the road to La Noria, Sinaloa, Mexico. work-up of the Me_2CO extracts of dry foliage gave, after chromatography on Si gel skimmianine, identified by its IR spectrum.

Isolation from Z. fagara. Plant material was collected at three sites; 45 miles south of Monterey, N. L. along Mexican highway #85 on the grade just south of the bridge over the Arroyo Garrapatas; along Mexican highway #190 *ca* 1 mile north of Nuevos Horizonles, Oaxaca, in Acateco canyon and 22 miles south of Mazatlan along Mexican highway #15. Work-up of the Me_2CO extracts of dry ground foliage gave, after chromatography on Si gel, Skimmianine eluted with C_6H_6 and scopoletin(6-methoxy-7-hydroxycoumarin) eluted with $CHCl_3$. Each was identical in properties with authentic samples.

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