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STUDIES OF ZOAPATLE I. THE EXTRACTION OF ZOAPATLE (*MONTANOA TOMENTOSA*) AND THE IDENTIFICATION OF 21-NORMONTANOL AS THE INITIAL DECOMPOSITION PRODUCT OF ZOAPATANOL

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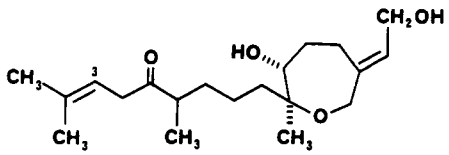
ABSTRACT.—Hot H_2O and hexane were found to be inappropriate to extract zoapatanol (**1**) from the leaves of *Montanoa tomentosa* ssp. *tomentosa*; EtOH and EtOAc, on the other hand, were far more effective. A number of *Montanoa* species and subspecies were examined for the presence of zoapatanol (**1**). Zoapatanol is highly unstable either neat or in aqueous solution at elevated temperatures, and only samples dissolved in solvent and stored at −5 to −10° have extended half-lives. The initial decomposition product of zoapatanol (**1**) has been identified as 21-normontanol (**2**) through high field proton nmr analysis.

The herbal tea brewed from leaves of the zoapatle plant, *Montanoa tomentosa* Cerv. (Compositae), has been used as a traditional medicine in Mexico for hundreds of years to induce labor and as an antifertility agent (1,2). A substantial number of papers and patents in the recent past have concerned the diterpene zoapatanol (**1**), one of the principles claimed to be responsible for the antiimplantation activity of the tea. The structure of **1** was established through X-ray crystallography of its tosylhydrazone derivative (3,4) and, subsequently, through total synthesis (5-7).



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Several human studies on the oral administration of a tea from zoapatle leaves have been reported (8-12). One of these reports indicated a luteolytic effect in normally menstruating women in the luteal phase (9), whereas the Swedish group (10) did not observe this effect, although distinct uterotonic and induction of cervical dilation effects were noted. Observations by the Ortho Pharmaceutical Company indicated that the tea possessed abortifacient, labor induction, and menstruation induction effects (8,11).

As part of our continuing studies on male and female fertility regulating agents from plants, we became interested in the detection, isolation, and storage stability of zoapatanol. Using a sample of synthetic (±)-zoapatanol[2] (13), we attempted to establish the most facile and efficacious procedure for the extraction of zoapatanol.

To determine the best extraction solvent, six extracts per solvent were prepared from 20-g leaf samples using the solvents C_6H_6, $CHCl_3$, EtOH, EtOAc, hexane, and

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[2]The sample of (±)-zoapatanol was received frozen in C_6H_6 for reasons which will become evident.

TABLE 1. Zoapatanol Concentration in Extracts Prepared from *Montanoa tomentosa* ssp. *tomentosa* Leaves Using Selected Solvents

Solvent	Zoapatanol Concentration (μg in 5.0 μl extract) Average S.E.
C_6H_6	0.26 (0)[a]
$CHCl_3$	0.33 (0.010)
EtOH	0.40 (0.015)
EtOAc	0.38 (0.011)
Hexane	-[b]
H_2O	T[c]

[a]Standard deviation; each extraction repeated six times, see Experimental section for details.

[b]Zoapatanol not detected by hplc analysis.

[c]T=Trace quantity detected, however, peak heights cannot be determined.

H_2O. Through hplc analysis on a μ-Bondapak C_{18} column, it was shown (Table 1) that EtOH or EtOAc were the preferred solvents, whereas H_2O and hexane extracted essentially *no* zoapatanol.

Analysis of the EtOAc extracts of the dried leaves of the seven species/subspecies of *Montanoa* cultivated at the University of Illinois at Chicago Pharmacognosy Field Station showed that *Montanoa leucantha* (Lag.) Blake ssp. *leucantha*, has the highest concentration of zoapatanol (0.0376%), followed by *M. tomentosa* ssp. *microcephala* (Sch.-Bip.) Funk at 0.0328%. *Montanoa laskowski* McVaugh was found to be devoid of zoapatanol in this study (Table 2).

TABLE 2. Zoapatanol Content of *Montanoa* Species and Varieties

Sample	Content (Per Cent)[a]
Montanoa laskowskii McVaugh	0[b]
Montanoa leucantha (Lag.) Blake ssp. *arborescens* (DC.) Funk	0.0176
Montanoa leucantha (Lag.) Blake ssp. *leucantha*	0.0376
Montanoa mollisima Brongn. ex Groenl.	0.0064
Montanoa tomentosa Cerv. ssp. *microcephala* (Sch.-Bip.) Funk	0.0328
Montanoa tomentosa Cerv. ssp. *tomentosa*	0.0184
Montanoa tomentosa Cerv. ssp. *xanthiifolia* (Sch.-Bip.) Funk	0.0232

[a]Represents an average analysis of four plant samples in duplicate, unless otherwise stated.

[b]Paucity of plant material permitted only two plant samples for analysis. The minimum quantity of zoapatanol detectable by the procedure used was 0.1 μg or 100 ng.

The neat sample of zoapatanol was studied for its stability on storage at 5°, at room temperature (25°), and at 55°. At the lower temperatures, samples were analyzed at 0, 12, 24, 48, 72, 128, 168, and 336 h; at 55°, samples were analyzed at 0, 1, 3, 6, 12, 18, 48, and 72 h. The half-lives established for zoapatanol under these sets of condi-

tions were 11.5 h (55°), 6.3 days, (25°, light) and 8.6 days (25°, dark). At 5°, about 20% decomposition was observed after 13.5 days. These data are shown graphically in Figure 1.

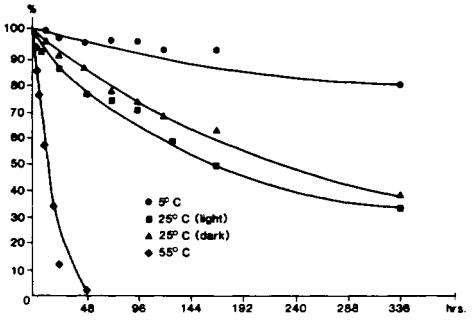


FIGURE 1. Stability of Zoapatanol (Neat) at 5, 25, and 55°.

Stability studies were also conducted on two solutions containing zoapatanol, H_2O, and EtOH. In the former case, the tea obtained after boiling the leaves for 15 min was filtered, concentrated in vacuo, and then spiked with zoapatanol. The extracts were stored at 5°, 25°, and 55° and the analyses conducted at 12, 24, 48, 72, 96, 120, 144, and 168 h (5° and 25° conditions) and at 1, 2, 4, 8, 12, 24, 36, and 48 h (55° conditions). The results are shown in Figure 2 for the EtOH extract and in Figure 3 for the aqueous extract. The stability of zoapatanol in the spiked tea was typically lower than that in the EtOH extract. At 55°, the half-life in the tea was about 6 h, whereas in EtOH it was about 78 h; at 25°, the half-life in the tea was 31 h and in EtOH, about 140 h. At 5°, the half-life in the tea was about 120 h. A 23% decomposition was observed after 168 h in EtOH at this temperature.

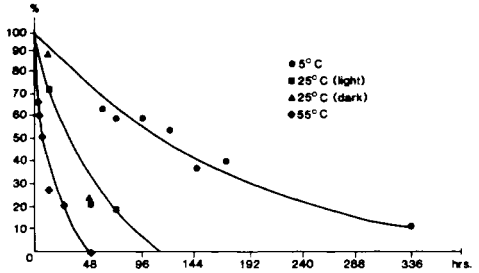


FIGURE 2. Stability of EtOH Extract of *Montanoa tomentosa* Leaves Stored at 5, 25, and 55°

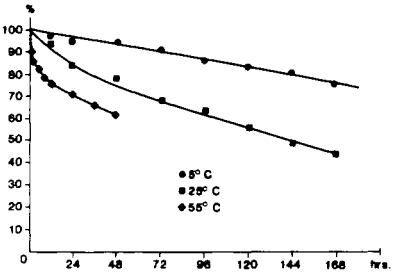
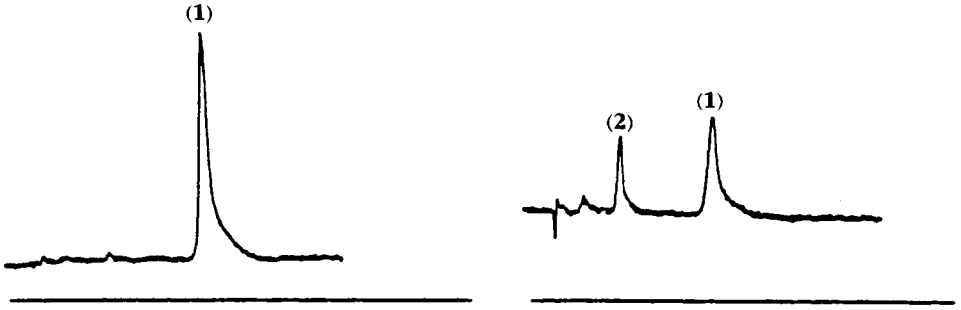


FIGURE 3. Stability of Spiked Aqueous Extract at 5, 25, and 55°

It was found that on exposure to light at room temperature for 72 h, significant conversion of (±)-zoapatanol to the decomposition product was observed (Figure 4). Preparatively, the conversion was induced by heating a sample of **1** on a water bath for 10 to 16 h at 40° or by keeping the sample at room temperature in the light for seven days. Although the analytical hplc separation was achieved on a $C_{18}\mu$-Bondapak reverse phase column, isolation was achieved through preparative tlc on silica gel (see Experimental). The isolate was stored under nitrogen and frozen in C_6H_6.

The chemical ionization mass spectrum established the decomposition product to have a M^+ at *m/z* 338, isomeric with zoapatanol (**1**), and the uv and ir spectra showing λ max 220 nm (log ε 3.95) and ν max 1685, 1655 cm^{-1}, respectively, suggested that the ketone carbonyl of **1** was now conjugated. Analysis of the 250 MHz ^{1}H-nmr spectrum



a) Sample stored in C_6H_6 (frozen) for 26 days.

b) Sample exposed to light at room temperature (25°) for 72 h.

FIGURE 4. Hplc Analysis of Stored Samples of Zoapatanol (**1**)

of the decomposition product on comparison with that of **1**, (Table 3) clearly indicated that the terminal methyl groups were now part of an isopropyl moiety, appearing as a doublet (J=16 Hz) at δ 1.37, and that the double bond had shifted to $\Delta^{3,4}$ since two *trans*-coupled (J=16 Hz) protons were observed at δ 6.90 (H-3) and δ 6.35. The two-proton triplet (4-H_2) observed at δ 3.15 in the spectrum of **1** was absent. The remaining signals were virtually the same as those observed in **1**. As expected (14), the base peak in the electron impact mass spectrum of the degradation product now appeared at

TABLE 3. ^{1}H-nmr Spectral Properties of Zoapatanol and Derivatives

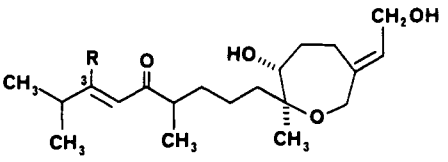
Proton	Compound			
	Zoapatanol[a] (**1**)	21-Normontanol[a] (**2**)	Tomexanthin[b] (**3**)	Montanol[c] (**4**)
1-H_3, 2-CH_3	1.63, 1.75 (s, 3H, each)	1.37 (d, J=7 Hz)	1.40 (s)	1.08 (d, J=7 Hz)
2-H	—	—	—	—
3-H	5.30 (t, J=7 Hz)	6.90 (dd, J=5, 16 Hz)	6.92 (d, J=15.5 Hz)	not given
3-CH_3	—	—	—	2.08 (d, J=2 Hz)
4-H	3.15 (d, J=7 Hz)	6.35 (d, J=16 Hz)	6.38 (d, J=15.5 Hz)	6.07 (bs)
6-H	2.60 (m)	2.85 (m)	—	not given
6-CH_3	1.08 (d, J=7 Hz)	1.10 (d, J=7 Hz)	1.12 (d, J=7 Hz)	1.08 (d, J=7 Hz)
10-CH_3	1.15, 1.16 (s, 1.5H, each)	1.16 (s)	1.15 (s)	1.13 (s)
11-H	3.56 (dd, J=6 Hz)	3.55 (m)	3.54 (dd, J=4, 8 Hz)	3.53 (br t)
13-H_2	2.24, 2.48 (m)	2.25, 2.45 (m)	2.75, 2.50 (dd, m)	
15-H	5.47 (t, J=6.5 Hz)	5.48 (t, J=7 Hz)	5.39 (t(br), J=7 Hz)	5.43 (m)
16-H_2	4.17 (bs)	4.22 (d, J=7 Hz)	4.60 (d, J=7 Hz)	4.13 (d, J=7 Hz)
17-H_2	4.10 (s)	4.12 (s)	4.12 (s)	4.10 (s)

[a]Obtained at 250 MHz on a Bruker WM-250 instrument.

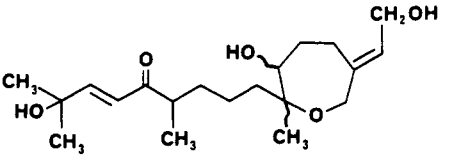
[b]Data are from Seaman *et al.* (16).

[c]Obtained at 60 MHz on a Varian T60A instrument, Levine *et al.* (1).

m/z 95, reflecting cleavage between C-5 and C-6 with the formation of a stable carbonyl-containing species. The decomposition product is therefore regarded as the 3,4-isomer of zoapatanol having the structure **2**. This compound may also be regarded as 21-normontanol. Although this compound has not formally been described in the primary literature, it is apparently contained within a patent without comment as to its physical and spectroscopic properties (15). A related compound, tomexanthin (**3**), has recently been isolated from *M. tomentosa* subsp. *xanthiifolia* (Schultz Bip. in C. Koch) Funk (16), and a comparison of its spectral data with those of **1**, **2**, and montanol (**4**) is presented in Table 3.



2 R=H
4 R=CH_3



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In summary, our results indicate that zoapatanol cannot be effectively extracted from the leaves of *M. tomentosa* ssp. *tomentosa* with hot H_2O, and that even if it could, it would be substantially decomposed. Secondly, our data suggest that either *M. leucantha* ssp. *leucantha* or *M. tomentosa* ssp. *microcephala* should be further investigated as a potential starting material for the extraction and isolation of zoapatanol. Thirdly, we have shown that zoapatanol should not be stored neat, but rather in an organic solvent at low temperature, and that 21-normontanol (**2**) is the primary decomposition product. These accumulated data lead us to suggest that zoapatanol may not be the sole agent responsible for the biological activities observed for the aqueous preparations of zoapatle leaves as has previously been reported (3,4,8,11).

EXPERIMENTAL

PLANT MATERIAL.—The plant materials used in this study were cultivated at our Pharmacognosy Field Station, of the University of Illinois at Chicago, located in Lisle, IL. Herbarium samples are deposited at the Field Museum of Natural History, Chicago, IL.

ORGANIC SOLVENT EXTRACTION OF PLANT MATERIAL.—A sample of the leaves of *M. tomentosa* ssp. *tomentosa* (20 g) was extracted with the organic solvent (300 ml) (hexane, C_6H_6, EtOAc, and EtOH) with constant stirring for 30 min at room temperature and the mixture filtered. This procedure was repeated twice and the final marc washed with solvent (100 ml) and the extracts combined. The organic phase was evaporated in vacuo below 40°, the residue dissolved in MeOH-acetonitrile (40 ml, 13:8), and the solution filtered through Kieselguhr (0.3 g). The volume of filtrate was made up to 40 ml through the addition of MeOH-acetonitrile (13:8) and the extract subjected to hplc analysis. This process was repeated on each of six plant samples for each solvent. The results are summarized in Table 1. EtOAc extracts were obtained for each of the plant materials listed in Table 2 by a similar process.

AQUEOUS SOLVENT EXTRACTION OF PLANT MATERIAL.—A sample of the leaves of *M. tomentosa* ssp. *tomentosa* (20 g) was extracted with boiling, distilled H_2O (200 ml) for 15 min. The solution was cooled, filtered, and concentrated in vacuo and then partitioned three times with EtOAc (100 ml each). The combined EtOAc extracts were dried (Na_2SO_4), filtered, and evaporated to dryness and the residue dissolved in MeOH-acetonitrile (40 ml, 13:8). The solution was filtered through Kieselguhr (0.3 g) and the volume of the filtrate made up to 40 ml through the addition of MeOH-acetonitrile (13:8). Hplc analysis was then conducted and the results are summarized in Table 1.

ANALYSIS FOR ZOAPATANOL.—The following solvent systems were found to be effective for the analysis of zoapatanol by tlc on silica gel 60 plates: Et_2O-MeOH (97:3), $CHCl_3$-acetonitrile (1:1), and acetonitrile-MeOH-H_2O (1:1:1) on C_{18}-impregnated silica plates.

High performance liquid chromatographic separation of the EtOAc extract was achieved on a μ-Bondapak C_{18} column (10 μ), using the solvent system MeOH-H_2O (45:55) and a flow rate of 1.5 ml/min. This system could not be used for the analysis of the hot H_2O extract of the leaves, and eventually the sys-

tem MeOH-acetonitrile-H_2O (13:8:40) was found to be most effective. In all instances, uv detection was accomplished at 214 nm. A precolumn of C_{18} Corasil was typically used, and analyses were performed in duplicate.

The apparatus used was a combination instrument consisting of a Beckman Model 241 system controller, Beckman Model 100A and Model 110A pumps, a Perkin-Elmer LC-85 variable wavelength detector, and an Altex Model C-RIA recorder.

ANALYSIS OF NEAT ZOAPATANOL SAMPLES.—Duplicate samples for each projected time of analysis were stored under the appropriate conditions [5°, 25° (dark), 25° (light), and 55°]. At the assigned time, the labeled sample and duplicate were removed from the stored environment, dissolved in acetonitrile, and made up to 10 ml with acetonitrile in a volumetric flask. Duplicate samples of 15 μl were then injected onto the hplc system from each flask. A μ-Bondapak C_{18} column (10μ particle size, 3.9 mm×30 cm) was used eluting with acetonitrile-H_2O (45:55) with a flow rate of 1.5 ml/min, detection at 214 nm, and a sensitivity of 0.04 Aufs; K′ was 4.2 for **1** and 1.7 for **2** with $\alpha=2.47$.

AQUEOUS EXTRACTION OF PLANT MATERIAL FOR STABILITY STUDIES.—A sample of the leaves of *M. tomentosa* ssp. *tomentosa* (20 g) was extracted with boiling H_2O (200 ml) for 15 min and the mixture cooled and filtered. The filtrate was concentrated in vacuo, refiltered and the filtrate (20 ml) mixed with zoapatanol (6 mg). From this spiked sample, aliquots were removed and stored under the following conditions 5° (dark), 25° (light and dark), and 55° (dark). Analyses were conducted at 12, 24, 48, 72, 96, 120, 144, and 168 h (5° and 25° conditions) and at 1, 2, 4, 8, 12, 24, 36, and 48 h (55° conditions) using the hplc system described above.

PREPARATION AND ISOLATION OF 21-NORMONTANOL (**2**).—Zoapatanol (20 mg) was heated at 40° on a water bath for 10-16 h or at room temperature for 7 days in the light. Chromatography of the reaction mixture was carried out on silica gel plates, prewashed successively with Me_2CO and acetonitrile-$CHCl_3$ (1:1), eluting with acetonitrile-$CHCl_3$ (1:1), twice. Two uv visible bands at Rf 0.35 (zoapatanol) and 0.25 (zoapatanol conversion product) were removed and extracted with Et_2O (60 ml). The Et_2O was removed at low temperature, and the extracts were dissolved in C_6H_6 and then frozen to reduce any further conversion. The zoapatanol conversion product **2** was obtained as a colorless oil exhibiting the following spectroscopic properties: ir ν max (neat) 3450, 2920, 2850, 1685, 1620, 1455, 1370, 1270, 1145, 1055, and 990 cm^{-1}; uv λ max (acetonitrile) 206 (log ϵ 4.18) and 220 nm (3.95); ^1H nmr, see Table 3; ms *m/z*, 70 eV 338 (M^+, not observed), 318 (5.2), 303 (2.5), 302 (1.2), 251 (4.3), 233 (2.5), 221 (93), 211 (19.1), 210 (51.3), 209 (78.8), 194 (3.7), 191 (12.4), 177 (4.5), 171 (3.7), 149 (27.2), 143 (6.2), 141 (20.9), 137 (24.7), 125 (23.5), 123 (28.4), 113 (80.3), 95 (100), 94 (52.5), 85 (51.9), 81 (73.5), 69 (38.3), 68 (82.8), 67 (80.3), and 55 (79.0).

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