ANTINEOPLASTIC AGENTS. 67. *SENECIO FENDLERI* GRAY[1]

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ABSTRACT.—A prominent anticancer constituent of the subalpine Rocky Mountain plant *Senecio fendleri* Gray was found to be the ethyl ester counterpart (**1a**) of Jacaranone. The structure of ethyl α-[4-(4-hydroxy-cyclohexa-2,5-dien-1-one)]-acetate (**1a**) was established by spectral (principally nuclear magnetic resonance and mass spectrometry) analysis and transformation to azobenzene **3**. An x-ray crystal structure determination of the latter substance (**3**) confirmed the overall assignments.

Among genera of the Compositae family *Senecio* is the largest and includes over 1,000 species. The *Senecio* genus is well known for its pyrrolizidine alkaloids and some of these exhibit a potentially useful level and spectrum of antitumor activity. However, use of these *Senecio* alkaloids has been limited due to hepatotoxicity and mutagenic properties (2). When we found an ethanol extract of *Senecio fendleri* Gray[2] (collected in Eastern Utah) to inhibit growth of the National Cancer Institute's P388 lymphocytic leukemia (3, PS system), isolation of the antineoplastic constituent(s) seemed of special interest at the time (1969) in respect to possibly uncovering a more useful *Senecio* biosynthetic product.

The aerial portion and roots of *Senecio fendleri* Gray collected in the flowering stage (June) were extracted successively with ligroin, ethanol and water. While the ligroin extract proved to be inactive against the PS system both the ethanol and water extracts gave T/C values of 130 at 50 mg/kg. The ethanol extract was selected for detailed separation directed by bioassay by deploying the sequence: chloroform-water partitioning, and separation of the chloroform fraction by gel permeation (Sephadex LH–20) followed by silica gel column chromatography. The major antineoplastic constituent obtained by this procedure was a colorless oil with the same relatively low order of activity (for example T/C 120 at 5 mg/kg) as the original extract. A complete structural assignment for the oily antineoplastic substance was established as follows.

Results of low resolution mass spectral determination combined with the infrared and proton magnetic resonance spectral data suggested either structures **1a** or **2** as reasonable possibilities. A sharp signal at 2.72 δ in the pmr spectrum seemed more consistent with the acetate methylene of structure **1a** rather than the methylene protons of primary alcohol **2** but initially the mass spectral fragmentation appeared somewhat more consistent with the latter interpretation. Also, the relatively easy acetylation of the oily ketone appeared more compatible with primary alcohol structure **2**. More compelling evidence favoring tertiary alcohol structure **1a** was provided by a high resolution mass spectrum (see chart 1) of acetate **1b**. Unequivocal evidence for the tertiary alcohol **1a** assignment was obtained during the course of evaluating potentially crystalline derivatives for x-ray crystallographic studies. Reaction of ketone **1a** with an acidic solution of 2,4-dinitrophenylhydrazine reagent proceeded to yield azobenzene **3**. The acid-catalyzed elimination of water from the tertiary alcohol (**1a**) and concomitant

[1]For the preceding contribution refer to Ref. (1).

[2]The plant occurs in dry forest through subalpine elevations in gravelly soils: See references 4 and 5.

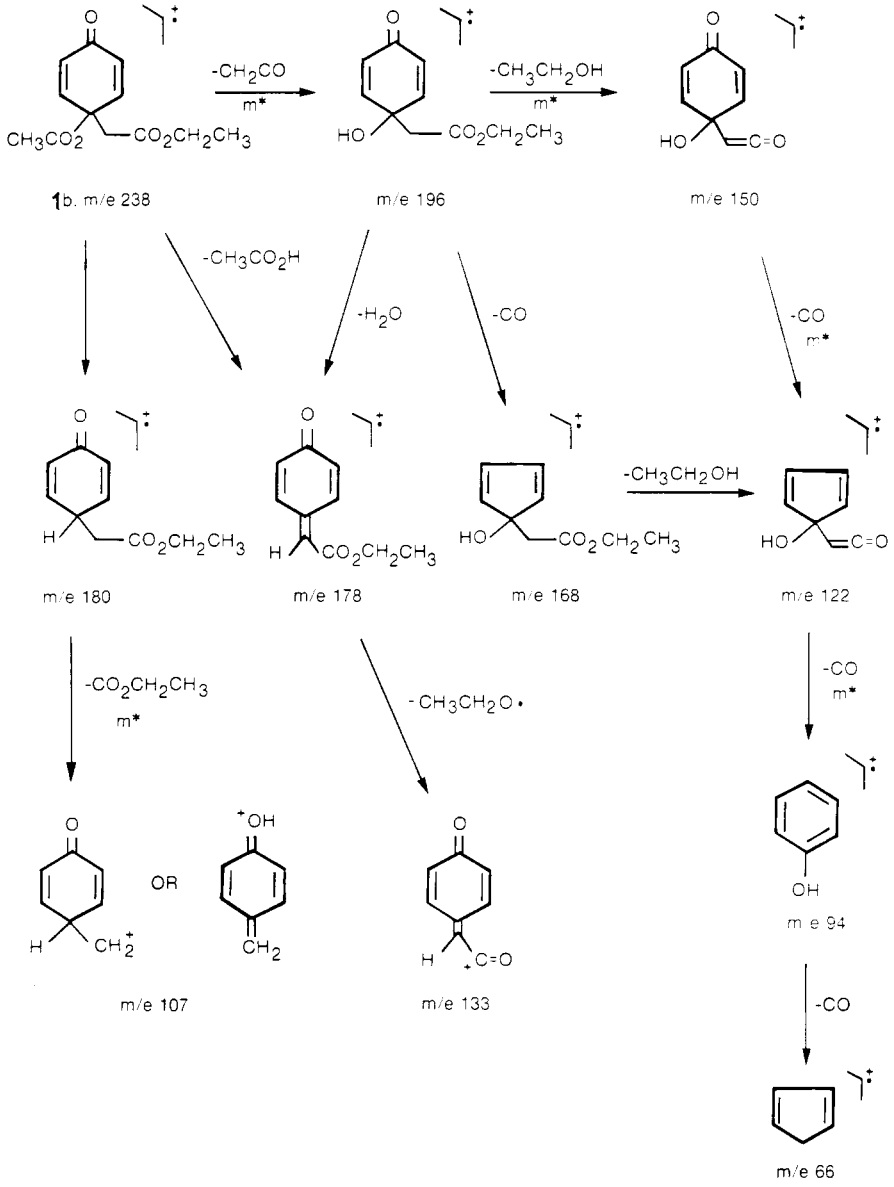
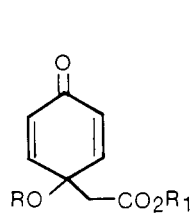


Chart 1

aromatization suggested by pathway **1a→3** is quite consistent with properties expected for ketone **1a**. An x-ray crystal structure analysis of azobenzene **3** confirmed the structure.

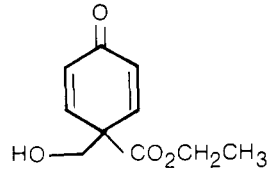
In a parallel investigation of the Columbian plant *Jacaranda caucuna* Pittier (Bignoniaceae) Farnsworth and colleagues (6) have isolated jacaranone (**1c**) as the PS active (T/C 165 at 2 mg/kg) component. Jacaranone was isolated as a pale yellow oil that slowly crystallized (mp 53–54°) and the structure was assigned



1a, R = H, $R_1 = CH_2CH_3$

b, $R = COCH_3$, $R_1 = CH_2CH_3$

c, R = H, $R_1 = CH_3$



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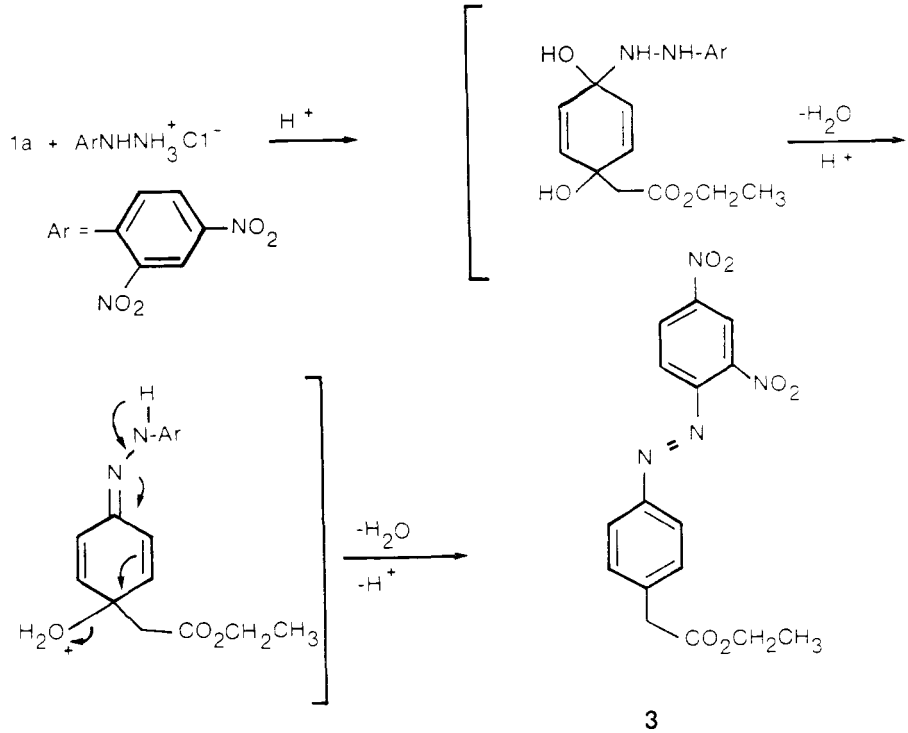

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on the basis of spectral evidence and base-catalyzed rearrangement to 2,4-dihydroxy-phenylacetic acid methyl ester. Recently, a total synthesis of jacaranone provided a specimen that upon recrystallization from ether-hexane melted at 80–81° (7).

Since a methanol extraction was employed to isolate jacaranone and ethanol was used in the present study one or both esters may have resulted from the plant extraction procedure. The actual natural product may be the corresponding carboxylic acid or a different ester. Interestingly, dienone **1a** appears to be only the third plant biosynthetic product of this type to be identified (6). The variable but definite life extension obtained against the murine PS leukemia using jacaranone

or its ethyl ester counterpart (**1a**) indicates that dienone **1a** probably accounts for most of the antineoplastic activity resulting from nonaqueous and aqueous extracts of *Senecio fendleri* Gray.

EXPERIMENTAL[3]

PLANT COLLECTION.—In June 1967, *Senecio fendleri* Gray in the flowering stage was collected (entire plant) in the Ashley National Forest (approximately 3 miles east of Summit Springs S. Guard Station) Utah. Recollections (again by GRP, M. J. Pettit, W. E. Pettit and M. S. Pettit) were made in June 1969 at the same location, (approximately 8,000 ft). Voucher specimens are maintained at the Cancer Research Institute, Arizona State University. The plant was divided into aerial and root portions and each was extracted separately. A series of ligroin, ethyl alcohol and water extracts of the roots did not display any significant activity against the P388 lymphocytic leukemia.

ISOLATION OF ETHYL α-[4-(4-HYDROXY-CYCLOHEXA-2,5-DIEN-1-ONE)]-ACETATE (**1a**).[4]—The flowers, leaves and stems (*ca.* 3 kg) of *Senecio fendleri* Gray were extracted with ligroin over a two-day period in a Soxhlet apparatus. The ligroin extract (inactive in the P388 system) was not further investigated. Continued exhaustive extraction with 95% ethyl alcohol gave upon concentration and lyophilization 204 grams of crude extract (PS T/C 130 at 50 mg/kg). A portion (26 g) of the crude ethyl alcohol extract was dissolved in chloroform (250 ml)-water (500 ml). The aqueous layer was separated and re-extracted with chloroform (3 x 108 ml) and the combined chloroform extract was concentrated to a 7.5 g residue (PS T/C 135 at 170 mg/kg). The corresponding water residue showed the same level of activity (PS T/C 135 at 160 mg/kg).

A solution of the chloroform fraction in methanol (30 ml) was filtered and applied to a column (5 x 85 cm) of Sephadex LH–20. The column was eluted with methanol at the rate of 40 ml per hr and the fractions corresponding to elution volumes 1.26–1.4 liters were combined to yield 1.07 g of crude ketone **1a**. The 1.07 g fraction was triturated with diethyl ether and the solution (0.78 g was soluble) was chromatographed on a column of silica gel (40g) employing a dry column technique (8). A fraction (0.21 g, 0.82% of the ethanol extract) eluted by diethyl ether corresponded to pure cyclohexa-2,5-dien-1-one **1a**; tlc (diethyl ether) Rf 0.37; glc (1 peak using 3% SE–30 on Chromasorb W at 130° with a ⅛ inch x 5 ft column). The ketone (**1a**) was obtained as a colorless oil (marginally active against PS showing T/C 120 at 5 mg/kg) which exhibited: ir (chloroform solution) νmax 3480, 3030, 2990, 1717, 1677, 1634, 1409, 1378, 1338 cm^{-1}; ^1H nmr δ, 1.25 (t, 3H, J=7.0 Hz), 2.72 (s, 2H), 4.18 (q, 2H, J=7.0 Hz), 4.3 (broad 1H, absent in deuteriomethanol), 6.15 (d, 2H, J=9.8 Hz), and 7.02 (d, 2H, J=9.8 Hz); ms *m/e* (relative intensity), 196 (M^+, 2.5%), 150 (14%), 122 (14%), 109 (54%), 107 (20%), 88 (36%), 43 (51%), 32 (46%), 28 (100%). The elemental composition was ascertained by results of high resolution mass spectrometry: calcd. for $C_{10}H_{12}O_4$, 196.07355; Found: *m/e* 196.07318 (M^+).

The acetate derivative (**1b**) was prepared by dissolving tertiary alcohol **1a** (25 mg) in pyridine (5 ml)-acetic anhydride (0.5 ml). After 3 days at room temperature the solvent was removed (rotary evaporation) and the residue dissolved in diethyl ether was chromatographed (dry column technique) on a column of silica gel. A fraction corresponding to pure acetate **1b** was eluted with the same solvent. The colorless oily acetate single spot and single peak by tlc, R_f 0.39, and glc (see above for methods) corresponded to the following spectral data: ir (neat) νmax 3480, 2982, 2939, 1740, 1673, 1634, 1370, 1225, 1180, 1025, 932, 857, 750 cm^{-1}; ^1H nmr 1.27 (t, 3H, J=7 Hz), 2.08 (s, 3H), 2.84 (s, 2H), 4.19 (q, 2H, J=7 Hz), 6.30 (d, 2H, J=10 Hz), 7.10 (d, 2H, J=10 Hz); ms *m/e* (relative intensity) 238 (very weak), 196 (41), 180 (31), 150 (35), 133 (26), 123 (17), 122 (17), 107 (56), 60 (23), 43 (53), 28 (100). To a solution of ketone

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[3]The Sephadex LH–20 and silica gel employed for column chromatography were supplied respectively by Pharmacia Fine Chemicals, Uppsala, Sweden and E. Merck, Darmstadt, West Germany. Thin layer chromatography was performed with E. Merck silica gel HF_{254} plates. The tlc chromatograms were developed by spraying and heating with 3% ceric sulfate in 10% aqueous sulfuric acid. The spectral data was obtained using the following instruments: Beckman IR–12, Perkin Elmer 299, Varian A–60 and XL–100 (deuteriochloroform solution with tetramethylsilane as internal standard); and Atlas MAT CH–4B and SM–1B mass spectrometers (electron impact at 70 ev). We wish to thank Miss K. Reimer and Messrs. E. Kelley and R. Scott for assistance with the spectral measurements.

[4]We wish to thank Mr. J. Day and Dr. R. M. Coomes for assistance with the extraction procedures.

1a (20 mg) in methanol (1 ml) was added 1.5 ml of a reagent prepared from 95% ethyl alcohol (10 ml), water (3 ml), concentrated sulfuric acid (2 ml) and 2,4-dinitrophenylhydrazine (0.40 g). After a 15 min period at room temperature the solid that separated was collected and recrystallized from ethyl alcohol-water to afford orange-red needles melting at 110–111°. The analytical specimen of azobenzene **3** was recrystallized from ethanol-ethyl acetate: mp 112–113°, ir (KBr)

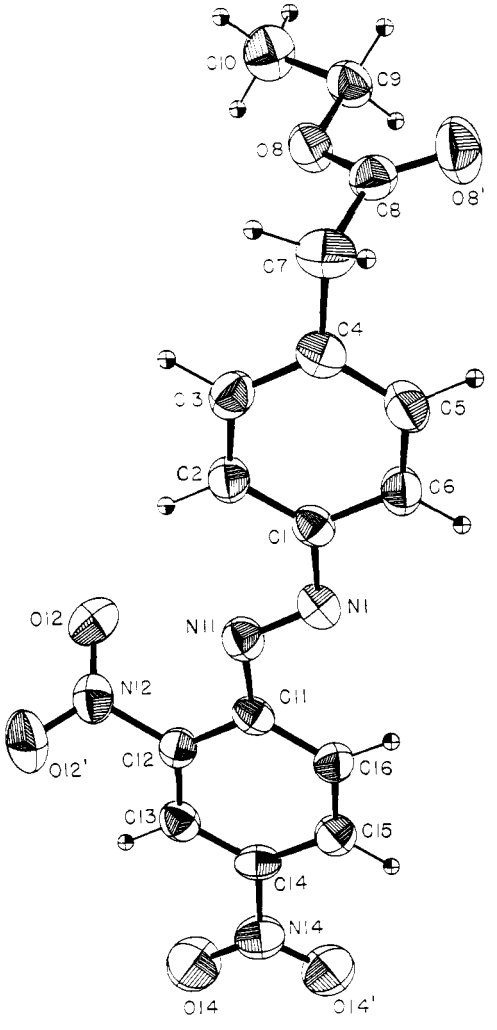

FIG. 1. Perspective view of the crystalline structure of ethyl α-[4-(4-hydroxycyclohexa-2,5-dien-1-one)] acetate (**3**); thermal ellipsoids for C, N and O are shown to include the 50% probability boundary surface. H atoms are represented as spheres with arbitrary radii (10).

νmax 3100, 2920, 1732, 1605, 1552, 1530, 1470, 1365, 1347, 1290, 1275, 1156, 1138, 1031, 870, 850, 830, 742, 721 cm^{-1}; ms *m/e* (relative intensity) 358 (M^+, 13%), 341 (1), 326 (3), 296 (1), 285 (64), 253 (72), 241 (2), 223 (2), 191 (33), 163 (100), 135 (15).

Anal. Calcd. for $C_{16}H_{14}N_4O_6$: C, 53.63; H, 3.94; N, 15.64; Found: C, 53.54; H, 3.92; N, 15.50.

X-RAY CRYSTALLOGRAPHIC ANALYSIS.[5]—Precession photographs of a single crystal of azobenzene **3** displayed diffractions consistent with the triclinic space group $P\bar{1}$ with $a=10.951(3)$ Å, $b=11.758(3)$ Å, $c=7.260(2)$ Å, $\alpha=106.50(1)°$, $\beta=91.68(1)°$, $\gamma=109.87(1)°$; $Z=2$; $\rho_{exp'l}=1.425$ g/cm^3 ($\rho_{calc'd}=1.431$ g/cm^3 for $C_{16}H_{14}N_4O_6$). Intensities of 3365 diffractions were measured in the θ-2θ variable scan speed mode on a Syntex $P\bar{1}$ autodiffractometer using graphite monochromated copper radiation. After correction for background, polarization and Lorentz

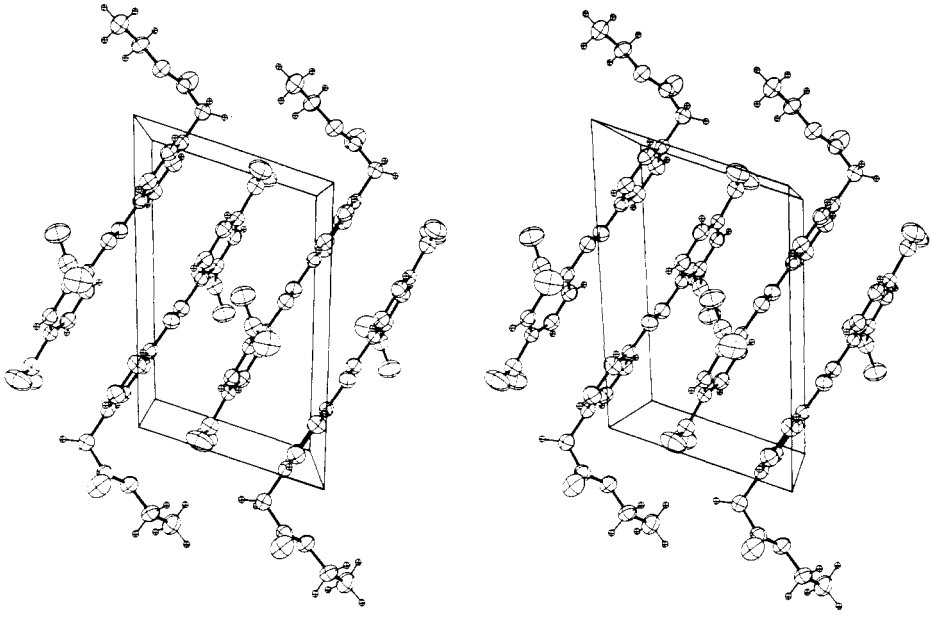

FIG. 2. Stereoscopic view of the molecular packing in the unit cell looking down the x axis.

effects, 3146 diffractions were considered observable. The structure was solved by direct methods using MULTAN (9). Large block least-squares refinement of the structure with anisotropic thermal parameters and fixed hydrogen atom positions converged at a standard residual $R=0.0432$ and a weighted residual $Rw=[\Sigma w(|F_o|-|F_c|)^2/\Sigma w|F_o|^2]^{1/2}=0.0411$ where $w=1/\sigma^2(F_o)$, using 1702 reflections restricted to $F_o \geq \sigma(F_o)$, $0.00<(\sin\theta)/\lambda \leq 0.51$ and $0<F_o<56.4$.

A perspective view (10), shown in figure 1, displays conformational aspects of azobenzene **3**. A stereoscopic crystal packing diagram presented in figure 2 reveals an overall planar stacking of the molecules. Interatomic bond lengths, bond angles and selected torsion angles are presented in figure 3.

ACKNOWLEDGMENTS

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[5]The x-ray structure factors are available from the authors.

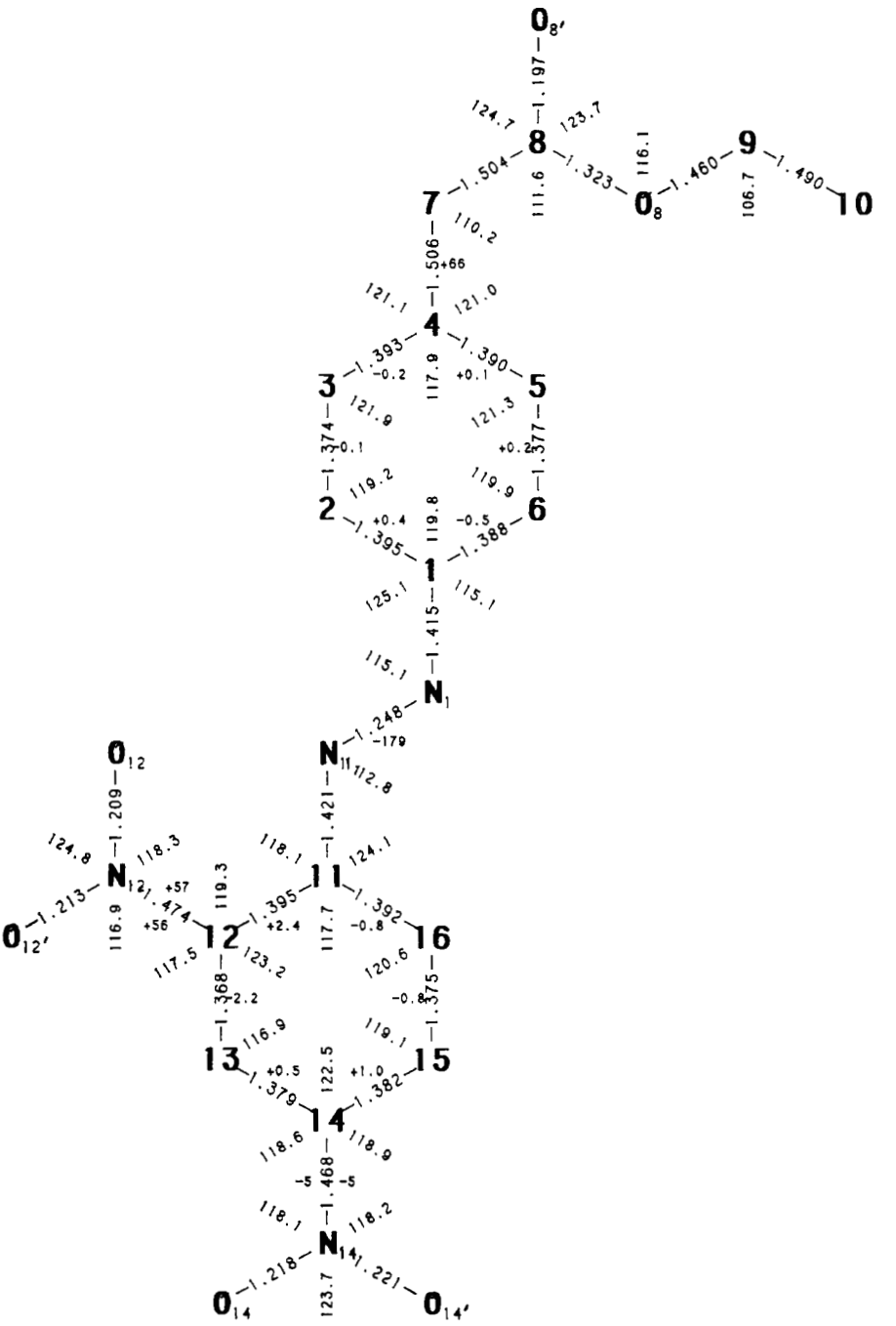

FIG. 3. Bond lengths (Å), bond angles (°), and torsion angles (°) in **3**.

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ERRATUM

For the paper entitled "The Antineoplastic Quassinoids of *Simaba cuspidata* Spruce and *Ailanthus grandis* Prain," Vol. 43, No. 4, p. 503, the third-named co-author should be Christian Moretti.