Eremophilane Sesquiterpenes from *Senecio Aureus*

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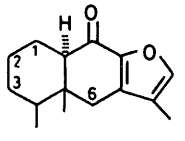
Three eremophilane sesquiterpenes have been isolated from *Senecio aureus*. *trans*-9-Oxofuranoeremophilane (1) has been previously isolated as a minor constituent of *Petasites hybridus*, while 8α-ethoxy-10α*H*-eremophilenolide (5) has not been previously reported. The structure of 3α-angeloyloxy-9-oxo-10α*H*-furanoeremophilane (7) was firmly established by a single crystal *X*-ray analysis and its spectral properties are consistent with the assigned structure. The previously reported spectral properties of 3α-angeloyloxy-9-oxo-10α*H*-furanoeremophilane are inconsistent with its structure.

THE genus *Senecio* of the tribe Senecioneae has long been known as a rich source of hepatotoxic pyrrolizidine alkaloids.[1] Only recently Bohlmann [2] has found that furanoeremophilanes are also abundant in this genus and in 1976 it was stated that over 100 such compounds had been isolated from the tribe Senecioneae.[3] Jennings [4] has now found that the hepatotoxins of *Tetradymia glabrata*, a member of the tribe Senecioneae, are also furanoeremophilanes.

Senecio aureus L. (golden ragwort), a common perennial was used by the Indians to facilitate childbirth [5-7] and is still part of the folk medicine of Appalachia.[8] Rather surprisingly, no systematic chemical work had been reported on this medicinal plant when we undertook this investigation, the only accounts being the rather confused reports of the presence of a pyrrolizidine alkaloid,[9] finally identified as senecionine.[10] Senecionine has shown some anti-cancer activity.[11,12] In view of the above, we undertook a chemical investigation of *Senecio aureus* and here report the isolation of three eremophilane sesquiterpenes from the 95% ethanol extract of the leaves. After we completed this investigation, Bohlmann *et al.*[2] reported the isolation of several furanoeremophilane sesquiterpenes, all of which are different from those reported here, from the roots and above ground parts of *S. aureus*. We will discuss the possible implications of these differences later in the paper.

Partition of the ethanol extract from the leaves of *Senecio aureus* between hexane and water yielded after evaporation of the hexane solvent a semisolid material. Chromatography on silica gel yielded in ether–hexane eluants three crystalline materials; compound A, m.p. 77—80 °C; compound B, m.p. 147—148 °C; and compound C, m.p. 158—160 °C.

Compound B was found to have a molecular formula of $C_{15}H_{20}O_2$ by exact mass determination. The i.r. and u.v. spectra (ν 1 660 cm^{-1}; λ 278 nm, log ε 4.0) indicated an oxo-group conjugated with a furan ring and the ^1H n.m.r. spectrum showed a methyl β (δ 2.02) and a single proton α to the oxygen (δ 7.35) of the furan ring, and two protons as an AB quartet next to the furan ring (δ 2.49 and 2.77, d, J = 16 Hz), a bridgehead methyl (δ 0.80), and a secondary methyl group (δ 0.93, d, J = 6 Hz). A search of the literature revealed that *trans*-9-oxofuranoeremophilane (1), previously isolated as a minor constituent in *Petasites hybridus* (*L.*) *Gaertn. Mey et Scherb*, rhyzomes,[13] possessed similar properties



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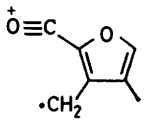

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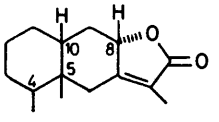

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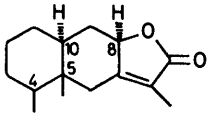


(4)

to compound B. We have had in hand authentic samples of both the *cis*- and *trans*-9-oxofuranoeremophilane † for comparison purposes, and copies of the i.r., n.m.r., and mass spectra of recently synthesized *trans*-9-oxofuranoeremophilane [3,‡] and compound B is definitely identical to *trans* and different from *cis*-9-oxofuranoeremophilane. The base peak in the mass spectrum of compound B appears at *m/e* 122. This is also the base peak in euryopsonol, and has been assigned to the fragment 2.[14]

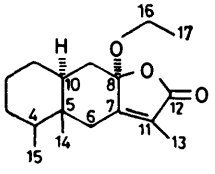
The molecular formula of compound A was found to be $C_{17}H_{26}O_3$ also by exact mass determination. The i.r. spectrum (ν 1 760 and 1 683 cm^{-1}) in conjunction with the u.v. spectrum (λ 222 nm, log ε 4.1) and the methyl signal at δ 1.85 in the n.m.r. spectrum indicated the presence of the typical α-methylbutenolide moiety. The nature of the two remaining sesquiterpenoid methyl groups was also evident from the n.m.r. spectrum which showed a methyl doublet at δ 0.91 and a high-field singlet at δ 0.58. These data were suggestive of an α-methyl-α,β-unsaturated-γ-lactone such as eremophilenolide [15] (3) except for three distinctive features.

First, the characteristic C-8 proton (δ 4.63) in eremophilenolide was absent from the spectrum of

† We are most grateful to Dr. L. L. Novotny, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Science, Prague, for copies of the n.m.r. spectra and authentic samples of *cis*- and *trans*-9-oxofuranoeremophilane.

‡ We thank Professor F. Bohlmann, Institut für Organische Chemie der Technischen Universität Berlin, Berlin for copies of the spectra of *trans*-9-oxoforanoeremorphilane.

compound A. Second, the ^1H n.m.r. spectrum showed the presence of a three proton triplet ($J = 7$ Hz) at δ 1.18 and a two proton quartet at δ 3.39 ($J = 7$ Hz) characteristic of an ethoxy-group, thus accounting for the extra oxygen and additional two carbons above the normal sesquiterpenoid lactone molecular formula. Also, it could be assumed that this ethoxy-group was attached at position C-8 [see structure (3)]. Third, the methyl signal at δ 0.58 was at too high field for a *cis* fused ring system such as that shown in (3) where the C-5 methyl appears at δ 1.03.[16] However, the *trans*-isomer 10-*epi*-eremophilenolide (4) has been prepared by hydrogenation of the natural ligularenolide [17] and its n.m.r. spectrum shows the C-5 methyl group at δ 0.58 and the C-4 methyl doublet at δ 0.91, the same positions observed for the methyl groups in compound A. Thus, structure (5) was tentatively assigned to compound A. The eremophilane skeleton rather than the eudesmane skeleton, as in (6), was chosen for compound A because in its n.m.r. spectrum, one of the two protons at C-6 [see (5)] appears as an AB doublet ($J = 14$ Hz) at δ 2.76, with the identical chemical shift and coupling constant to that of one of the C-6 protons of compound (4) [16] [the other C-6 proton in both compound A and in (4) is buried further up field]. If structure (6) had been



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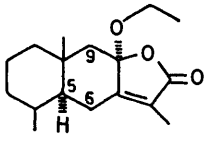
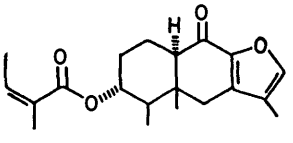


(6)

correct, neither C-6 proton would have appeared simply as an AB doublet but rather as a ABX system with further coupling to the C-5 proton. Finally, the ^{13}C n.m.r. spectrum of compound A gave additional support for structure (5). Thus, in the off-resonance decoupled spectrum, the lowest field singlet at δ 171.0 could be assigned to carbonyl carbon C-12, the singlet at δ 157.4 could be assigned to C-11, the singlet at δ 124.4 to C-7, and the singlet at δ 105.8 could be assigned to C-8. The only other singlet in the spectrum appeared at δ 40.7 and this could be assigned to C-5. A triplet at δ 58.0 could be assigned to C-16. In addition, the ^{13}C n.m.r. spectrum showed four high-field quartets and six triplets as expected for structure (5). The ethyl group found in compound A may be an artifact caused by the ethanol extraction solvent. If this is the case then the actual compound present in the plant is 10α*H*-8-hydroxyeremophilenolide, the *cis*-isomer of which has previously been synthesized by an oxidation of *cis*-furanoeremophilane with oxygen.[17]

The n.m.r. spectrum of compound C suggested a furanoeremophilane skeleton. Thus the vinyl proton at δ 7.37 (q, $J = 1.2$ Hz), methyl singlet at δ 0.88, methyl doublet ($J = 6.6$ Hz) at δ 1.00, methyl doublet ($J = 1.2$ Hz) at 2.00, and the C-6 AB quartet ($J = 16.3$ Hz) at δ 2.50 and 2.77 were almost identical to those seen in compound B. Likewise the i.r. and u.v. spectra of compound C (ν 1 675 cm^{-1}, $\lambda_{max.}$ 280, log ε 5.05) indicated that it was a 9-oxofuranoeremophilane. This information together with a molecular formula of $C_{20}H_{26}O_4$ suggested the presence of an angeloyloxy-group. This was confirmed from the n.m.r. spectrum which contained a methyl doublet ($J = 7.5$ Hz) at δ 1.98 and a vinyl quartet of quartets ($J = 1.5$, 7.4 Hz) at δ 6.00. The i.r. and u.v. spectra (ν 1 700 cm^{-1}, $\lambda_{max.}$ 217, log ε 3.98) were also consistent with an angelate ester. The n.m.r. spectrum indicates that the angeloyloxy-group was attached to a secondary carbon. The hydrogen on this carbon appeared as a doublet of triplets ($J = 4.0$ and 10.5 Hz) indicating that the site of attachment was either at carbon 1 or carbon 3.

Comparison of the data for compound C with that previously reported for 3α-angeloyloxy-9-oxofuranoeremophilane (7) [18] showed a great deal of similarity. However, the reported i.r. and u.v. spectra were different and were in fact inconsistent with structure (7). It has been reported that one can distinguish between 6-oxo- and 9-oxo-furanoeremophilane by their u.v. spectra.[14] Thus 6-oxo-compounds have a $\lambda_{max.}$ at 269 nm and 9-oxo at 280 nm. This information together with the reported u.v. of 269 nm would then lead one to believe that the previously reported material was 3α*H*-angeloyloxy-6-oxofuranoeremophilane (8) rather than the 9-oxo-material (7). The i.r. spectrum for the angelate ester function also appears to be inconsistent with structure (7). The absorption for an α,β unsaturated ester should be 1 700 cm^{-1} as compared to the reported absorption of 1 730 cm^{-1}. These inconsistencies made it difficult to identify positively compound C by comparison with the reported data. Fortunately compound C crystallized in a form suitable for *X*-ray analysis and we now know conclusively that the angeloyloxy-group is attached to carbon 3 and that compound C has structure (7) (see Figure).



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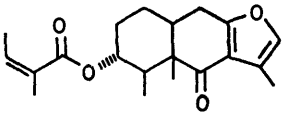


(8)

A crystal of compound C with the approximate dimensions 0.7 × 0.5 × 0.5 mm was mounted on a glass fibre using epoxy cement such that the longest crystal dimension was approximately parallel to the fibre axis.

Unit-cell parameters and the orientation matrix were determined on a Syntex $P2_1$ four-circle diffractometer equipped with a graphite monochromator using Mo-K_α radiation. Unit cell parameters obtained were $a =$ 12.279(5) Å,* $b = 7.680(3)$ Å, $c = 19.724(5)$ Å, and $V = 1\,860(2)$ Å^3. The crystal belonged to the orthorhombic system and space group $P2_12_12_1$ (No. 19) [19] was uniquely determined by systematic absences.

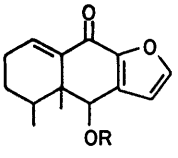
* Numbers in parentheses here and elsewhere in this paper indicate estimated standard deviations in the least significant digit(s).

Intensity data were collected using $\theta - 2\theta$ scans with X-ray source and monochromator settings identical to those used for the determination of the unit cell parameters. No significant fluctuations were observed in the intensities of three standard reflections monitored every 97 reflections. From a total of 1 931 unique reflections collected out to $2\theta = 50°$, 1 268 were accepted as statistically above background on the basis that I was greater than $3\sigma(I)$.

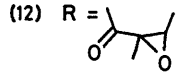
Computations were performed using standard programs.* For structure factor calculations the scattering factors were taken from Cromer and Waber's tabulation [20] for all atoms except hydrogen; Stewart's hydrogen atom scattering factors [21] were used. The agreement factors were defined in the usual way as $F = (\Sigma||F_o| - |F_c||)/(\Sigma|F_o|)$ and $R_w = [\Sigma_w(|F_o| - |F_c|)^2/\Sigma_w(|F_o|)^2]^{\frac{1}{2}}$. In all least-squares refinements, the quantity minimized was $w(|F_o| - |F_c|)^2$. A weighting scheme based on counting statistics [$w = 4I/\sigma(I)^2$] was employed for calculating R_w in the least-squares refinement.

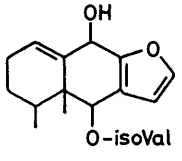
All non-hydrogen atoms were located from an E-map based on phases generated by MULTAN. Hydrogens were located from difference Fouriers and calculations based on ideal geometry after several cycles of full-matrix least-squares refinement. The final parameters varied included an overall scale factor, positional parameters for the oxygens and carbons, anisotropic thermal parameters for the oxygens, and isotropic thermal parameters for the carbons (117 variables; 1 268 observations). The hydrogen positions were not varied and their isotropic thermal parameters were fixed at 5.0. The final R factor was 0.081 and $R_w = 0.085$.†

As previously mentioned, Bohlmann *et al.*[2] just reported the isolation of the furanoeremophilanes (9)—



(9) R = Ac
(10) R = Ang
(11) R = Bu^i
(12) R =





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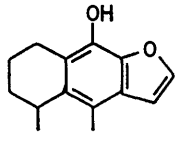

(14)

(14) and cacalol (13) from the roots and above ground parts of *Senecio aureus* obtained from the Botanical Gardens of Copenhagen, Denmark. We do not at this time, know why our plant material (specifically leaves), collected at Merrimac Mine, Montgomery County, Virginia, U.S.A. was found to contain the three different eremophilane sesquiterpenes (1), (5), and (11) and none of those identified by Bohlmann *et al.*[2] (isolated from roots and/or total above ground plant material), and it is particularly intriguing that all of Bohlmann's compounds are oxygenated at C-6 [except for cacalol (13), which undoubtedly arises from a C-6 oxygenated precursor] while none of those isolated by us are oxygenated at C-6. These differences may be of biosystematic significance.

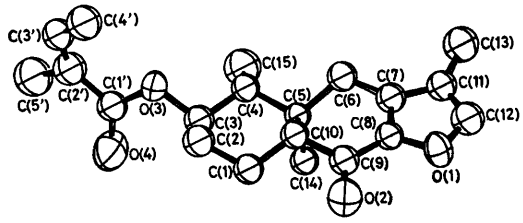

TABLE 1

Co-ordinates of the oxygen atoms (standard deviations are indicated in parenthesis and refer to the last decimal place)

Atom	X	Y	Z
O(1)	−0.082 3(4)	0.341 7(7)	0.479 4(3)
O(2)	−0.286 0(4)	0.532 4(9)	0.478 0(3)
O(3)	−0.278 8(5)	1.081 8(7)	0.722 7(3)
O(4)	−0.312 0(8)	1.336 8(9)	0.675 1(4)

TABLE 2

Co-ordinates of the carbon atoms (standard deviations are indicated in parenthesis and refer to the last decimal place)

Atom	X	Y	Z	B
C(1)	−0.325 8(7)	0.803 3(11)	0.569 0(4)	5.0(2)
C(2)	−0.354 6(7)	0.907 8(12)	0.631 6(4)	5.6(2)
C(3)	−0.252 2(7)	1.004 6(12)	0.656 7(4)	4.8(2)
C(4)	−0.157 4(6)	0.885 4(11)	0.668 2(4)	4.4(2)
C(5)	−0.125 9(5)	0.773 4(10)	0.603 9(4)	3.4(1)
C(6)	−0.040 5(6)	0.636 5(11)	0.523 7(4)	4.8(2)
C(7)	−0.029 6(6)	0.499 2(11)	0.669 6(4)	4.8(2)
C(8)	−0.110 4(6)	0.469 4(10)	0.524 1(4)	4.1(1)
C(9)	−0.214 7(7)	0.556 4(10)	0.521 8(4)	4.6(2)
C(10)	−0.231 3(6)	0.679 2(10)	0.581 7(4)	4.2(2)
C(11)	0.055 6(6)	0 386 4(11)	0.553 7(4)	4.8(2)
C(12)	0.018 6(8)	0.297 9(11)	0.498 8(5)	5.7(2)
C(13)	0.162 5(8)	0.374 1(14)	0.590 7(5)	6.6(2)
C(14)	−0.085 9(6)	0.890 0(10)	0.547 9(4)	4.3(2)
C(15)	−0.059 9(8)	0.989 2(15)	0.694 7(5)	7.1(2)
C(1′)	−0.307 0(7)	1.247 2(12)	0.724 8(4)	4.9(2)
C(2′)	−0.333 4(8)	1.309 6(12)	0.793 4(4)	5.9(2)
C(3′)	−0.386 1(7)	1.218 1(12)	0.839 3(4)	5.3(2)
C(4′)	−0.422 4(8)	1.039 4(14)	0.836 3(5)	6.6(2)
C(5′)	−0.305 2(9)	1.506 9(16)	0.807 1(6)	8.0(3)

EXPERIMENTAL

M.p.s were taken on a Kofler hot-stage and are uncorrected. I.r. spectra were recorded with a Perkin-Elmer 237 B spectrophotometer. ^{1}H N.m.r. spectra were obtained with a Varian A-60D or T60 spectrometer using Me_4Si as an internal standard (δ 0); ^{13}C n.m.r. spectra were run on a JEOL-PFT-100 FT spectrometer. Mass spectra were run on a Hitachi RMV-7 spectrometer and gas chromatography was done with a F & M Biomedical Gas Chromatograph, model 402.

Extraction and Preliminary Separation.—Approximately

* Programs utilized were Zalkin's FORDAP Fourier summation program, Iber's NUCLS modification of the Busing-Martin-Levy least-squares program, and Johnson's ORTEP program.

† The structure factors and thermal parameters are deposited as a supplementary publication (Sup. No. 22474, 9 pp.) For details of the Supplementary Publication Scheme, see Notice to Authors No. 7, *J.C.S. Perkin I*, Index issue, 1978.

TABLE 3

Co-ordinate of the hydrogen atoms

Atom	*X*	*Y*	*Z*	*B*
H(1A)	−0.40	0.74	0.56	5.0
H(1B)	−0.31	0.88	0.53	5.0
H(2A)	−0.12	0.16	0.18	5.0
H(2B)	−0.08	−0.02	0.12	5.0
H(3)	−0.24	0.10	0.62	5.0
H(4)	−0.20	0.82	0.70	5.0
H(6A)	0.02	0.70	0.64	5.0
H(6B)	−0.08	0.58	0.66	5.0
H(10)	−0.25	0.61	0.62	5.0
H(12)	0.06	0.22	0.47	5.0
H(13A)	0.16	0.34	0.64	5.0
H(13B)	0.21	0.30	0.57	5.0
H(13C)	0.20	0.49	0.59	5.0
H(14A)	−0.14	0.95	0.53	5.0
H(14B)	−0.04	0.97	0.57	5.0
H(14C)	−0.05	0.82	0.51	5.0
H(15A)	−0.02	1.05	0.66	5.0
H(15B)	−0.09	1.07	0.73	5.0
H(15C)	−0.01	0.91	0.72	5.0
H(3′)	−0.40	0.30	0.88	5.0
H(4′A)	−0.50	0.00	0.82	5.0
H(4′B)	−0.37	−0.02	0.81	5.0
H(4′C)	−0.41	0.00	0.88	5.0
H(5′A)	−0.38	0.58	0.78	5.0
H(5′B)	−0.30	0.48	0.86	5.0
H(5′C)	−0.24	0.53	0.78	5.0

1 lb (450 g) of freshly collected, air dried leaves of *Senecio aureus L.** was macerated in a Waring Blender with 95% EtOH and continuously extracted with 95% EtOH (3 l) for 2 d. Removal of the solvent with a rotary evaporator left 86.9 g of a dark residue. This residue was partitioned between water (1 l) and hexane (1 l) and, after drying ($MgSO_4$) and evaporation of the hexane left 22.5 g of residue. The residue (2 g) was chromatographed on silica gel 60 (EM Reagents, 230—400 mesh ASTM) (120 g) in a column approximately 2.5 × 50 cm. Material was initially eluted with hexane and then with increasing amounts of diethyl ether in hexane; 55 100-ml fractions were taken. Fraction 23, eluted in hexane–ether (9 : 1) yielded 0.121 g of material later identified (see below) as 8α-ethoxy-10α*H*-eremophilenolide (5). Fractions 28 and 29, also eluted in hexane–ether (9 : 1) yielded 0.045 g of material identified (see below) as *trans*-9-oxofuranoeremophilane. Fraction 39, eluted in hexane–ether (8 : 2), crystallized with time to give 0.105 g of material identified, as described below, as 3α-angeloyloxy-9-oxo-10α*H*-furanoeremophilane (14).

8α-*Ethoxy*-10αH-*eremophilenolide* (5).—In order to obtain more material for structure elucidation, a larger scale chromatography was undertaken as follows. The above mentioned hexane extract (10 g) was chromatographed on silica gel (Grace, grade 923, 100—200 mesh) (250 g). Elution with hexane–ether (9 : 1) gave several fractions, identical by g.l.c. and showing a single peak, which were combined, the solvent removed with a rotary evaporator, and distilled to give a colourless liquid (0.156 g), b.p. 185 °C/0.30 mmHg (air-bath). The oil crystallized at room temperature after 2 months to give material with m.p. 77—80 °C, mass spectroscopic molecular weight 278.188 18 ± 20 p.p.m. (Calc. for $C_{17}H_{26}O_{31}$ 278.188 20); $\nu_{max.}$(CHCl$_3$) 1 760 cm^{-1}; $\lambda_{max.}$(EtOH) 222 nm (ε = 13 725); ^{1}H n.m.r. δ(CDCl$_3$) 0.58 (3 H, s), 0.91 (3 H, d, J = 5), 1.18 (3 H, t, J = 7), 1.85 (3 H, d, J = 1.5), 2.7 (1 H, d, J = 14), 3.39 (2 H, q, J = 7), 1.2—2.4 (11 H); ^{13}C n.m.r. δ(CDCl$_3$) 8.1 (q), 10.8 (q), 15.3 (q), 15.5 (q), 26.0 (t), 28.0 (d), 30.5 (t), 37.6 (t), 40.5 (t), 40.7 (s), 41.9 (t), 42.8 (d), 58.0 (t), 105.8 (s), 124.4 (s), 157.4 (s), and 171.0 (s); M^+ *m/e* 278 (58%), 235 (28), 205 (55), 126 (25), 123 (25), 109 (100), 95 (28), 81 (35), 67 (31), 55 (37), and 53 (28).

trans-9-*Oxofuranoeremophilane* (1).—Continued elution of the silica gel with ether–hexane (9 : 1), as described above for the isolation of (5) gave several fractions particularly rich in a single component as detected by gas chromatography on a 5% SE 30 column. These combined fractions (0.64 g) were rechromatographed on 25 g silica gel 60 and yielded pure *trans*-9-oxofuranoeremophilane (1) (0.161 g) in the hexane–ether eluant (9 : 1), m.p. 147—148 °C (from benzene–hexane) (lit.,[13] 140—149 °C, lit.,[3] 146 °C). Mass spectroscopic molecules weight 232.149 2 ± 20 p.p.m. (Calc. for $C_{15}H_{20}O_2$: 232.146 3); $\nu_{max.}$(CHCl$_3$) 1 660 cm^{-1}, lit.,[13] 1 660 cm^{-1}; $\lambda_{max.}$(EtOH) 278 nm (log ε 4.0), lit.,[13] 278 (log ε 4.1); δ(CDCl$_3$) 0.80 (3 H, s), 0.93 (3 H, d, J = 6), 2.02 (3 H, s), 2.49 (1 H, d, J = 16), 2.77 (1 H, d, J = 16), 1.2—2.4 (8 H), 7.4 (1 H, m); M^+ *m/e* 232 (78%), 217 (96), 175 (39), 161 (57), 149 (25), 123 (56), 122 (100), 109 (60), and 108 (29).

3α-*Angeloyloxy*-9-*oxo*-10α*H*-*furanoeremophilane* (7).—The crystalline (0.105 g) 3α-angeloyloxy-9-oxo-10α*H*-furanoeremophilane described above under *Extraction and preliminary separation* was recrystallized by the slow evaporation of an ether solution to give material of m.p. 158—160 °C (Found: C, 72.73, H, 7.97. Calc. for $C_{20}H_{26}O_4$: C, 72.68; H, 7.94); $\nu_{max.}$(CHCl$_3$) 1 675 and 1 700 cm^{-1}; $\lambda_{max.}$(EtOH) 280 nm (ε 11 300) and 217 nm (ε 9 615); δ(CDCl$_3$) 0.88 (3 H, s), 1.00 (3 H, d, J = 6.6), 1.91 (3 H, d, J = 1.5), 1.98 (3 H, d, J = 7.5), 2.00 (3 H, d, J = 1.2), 2.13—2.47 (6 H), 2.50 (1 H, d, J = 16.3), 2.77 (1 H, d, J = 16.3), 4.84 (1 H, d of t, J = 4, 10.5), 6.00 (1 H, q of q, J = 7.4, 1.5), and 7.37 (1 H, q, J = 1.2); M^+ *m/e* 330 (2%), 162 (45), 83 (48), 66 (25), 55 (100), 53 (55), and 43 (50).

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REFERENCES

[1] L. B. Bull, C. C. J. Culvenor, and A. T. Dick, 'The Pyrrolizidine Alkaloids,' North-Holland Publishing Company, Amsterdam, 1968.
[2] F. Bohlmann, K-H. Knoll, C. Zdero, P-K. Mahanta, M. Grenz, A. Suwita, D. Ehlers, N-L. Van, W-R. Abraham, and A-N. Natu, *Phytochemistry*, 1977, **16**, 965, and references therein.
[3] F. Bohlmann, H. J. Forster, and C-H. Fischer, *Annalen*, 1976, 1487.
[4] P. W. Jennings, J. C. Hurley, S. K. Reeder, A. Holian, P. Lee, C. N. Caughlan, and R. D. Larsen, *J. Org. Chem.*, 1976, **41**, 4078, and references therein.
[5] A. Krochmal and C. Krochmal, ' A Guide to the Medicinal Plants of the United States,' Quadrangle/The New York Times Book Co., 1973, p. 204.
[6] C. F. Millspaugh, ' American Medicinal Plants,' John C. Yorston & Co., Philadelphia, 1892, Dover Publications, Inc., 1974, p. 357.
[7] W .H. Lewis and M. P. F. Elvin-Lewis, ' Medical Botany,' John Wiley & Sons, New York, 1977, p. 322.
[8] A. Krochmal, R. S. Walters, and R. M. Doughty, ' A Guide to Medicinal Plants of Appalachia,' Agriculture Handbook No. 400, U.S. Department of Agriculture, 1971.
[9] R. H. F. Manske, *Canad. J. Res.*, 1936, **14B**, 6.
[10] R. H. F. Manske, *Canad. J. Res.*, 1939, **17**, 1.

[11] W. H. Lewis and M. P. F. Elvin-Lewis, ' Medical Botany,' John Wiley & Sons, New York, 1977, p. 135.
[12] C. C. J. Culvenor, *J. Pharm. Sciences*, 1968, **57**, 1112.
[13] L. Novotny, J. Jezba, V. Herout, and F. Sorm, *Coll. Czech. Chem. Comm.*, 1962, **27**, 1393; L. Novotny, Z. Samek, J. Harmatha, and F. Sorm., *Coll. Czech. Chem. Comm.*, 1969, **34**, 336.
[14] D. E. A. Rivett and G. R. Woolard, *Tetrahedron*, 1967, **23**, 2431.
[15] L. Novotny, J. Jizba, V. Herout, F. Sorm, L. H. Zalkow, S. Hu, and C. Djerassi, *Tetrahedron*, 1963, **19**, 1101.
[16] E. Piers and M. B. Geraghty, *Canad. J. Chem.*, 1973, **51**, 2166.
[17] L. Novotny, V. Herout, and F. Sorm, *Coll. Czech. Chem. Comm.*, 1964, **29**, 2182.
[18] F. Bohlmann, C. Zdero, and M. Grenz, *Chem. Ber.*, 1974, **107**, 2730.
[19] ' International Tables for X-ray Crystallography, ' Vol. I, Kynoch Press, Birmingham, England, 1952.
[20] D. T. Cromer and J. T. Waber, *Acta Cryst.*, 1965, **18**, 104.
[21] R. F. Stewart, E. R. Davidson, and W. T. Simpson, 1965, *J. Chem. Phys.*, **42**, 3175.