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REPIN, A SESQUITERPENE LACTONE FROM *ACROPTILON REPENS* POSSESSING EXCEPTIONAL BIOLOGICAL ACTIVITY

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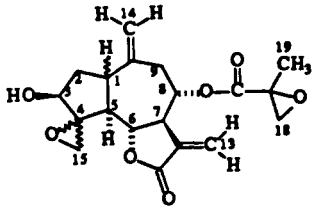
ABSTRACT.—Consumption of Russian knapweed (*Acroptilon repens*) by horses results in the necrosis of neural cells in the substantia nigra. Repin [**5**], one of the sesquiterpene lactones found in Russian knapweed, has been shown to possess high toxicity toward chick embryo sensory neurons. The possible causal relationship between repin and equine nigropallidial encephalomacia disease prompted a more complete structural assignment of repin, which was accomplished by X-ray and ^{1}H-nmr analyses.

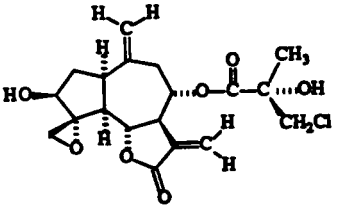
Acroptilon repens L. (*Centaurea repens*, Russian knapweed) is a fast growing perennial weed of the family Asteraceae which is rapidly becoming a major problem in many parts of the United States. This introduced weed has few if any of the natural enemies normally found in its country of origin. As a consequence, Russian knapweed is spreading aggressively, rendering agricultural land and rangeland useless (1).

Russian knapweed has also been implicated as the causative agent in a nervous system disease in horses called equine nigropallidal encephalomalacia (ENE) (2), a disorder characterized by the necrosis and softening of specific brain tissue (3), with symptoms not unlike Parkinson's disease, i.e., staggering, lip twitching, involuntary chewing movements, and other neurological symptoms.

A large number of sesquiterpene lactones, many of which are cytotoxic, have been isolated from the family Asteraceae (4), and in particular several have been found in Russian knapweed. The guaianolide, repin, was first isolated from Russian knapweed and characterized as **1** by Evstratova *et al.* in 1972 (5). In 1976 Gonzalez *et al.* (6) isolated repin together with several other sesquiterpene lactones and revised its structure to **2**. This was followed by an investigation of Russian knapweed by Rustaiyan *et al.* (7) in 1981 in which they also reported the structure of repin as **2**; however, they did not discuss their reasons for assigning a cis fusion of the carbocyclic ring systems. To further confuse the structural assignment, Mompen and Toubiana (8) isolated a compound, subluteolide, from *Vernonia sublutea* and also assigned it structure **2**. However, comparison of its spectral data with that of repin shows many similarities but some distinct differences, suggesting an isomeric relationship. Due to the uncertainties surrounding the structure of repin and other related compounds, Ste-



1 H-1=β; 4,15-epoxide=β
2 H-1=α; 4,15-epoxide=β
3 H-1=α; 4,15-epoxide=α



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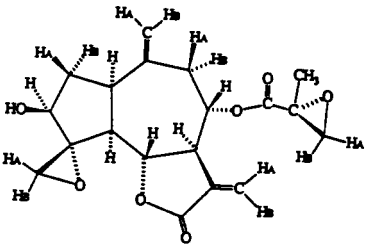
vens (9) investigated the structural assignments of repin and other sesquiterpenes from Russian knapweed and concluded that repin was best depicted as **3**. It was also concluded that subluteolide differed from repin by being epimeric at C-17. The absolute configuration of the side chain was not, however, determined at that time. The absolute configuration of acroptilin [**4**], another guaianolide isolated from Russian knapweed, was determined by X-ray crystallography in 1982 by Stevens and Wong (10) and suggests by biosynthetic analogy that the absolute configuration of C-17 in repin is *R*, the same as acroptilin.

Repin [**3**] was isolated from an Me_2CO extract of dried, ground aerial parts of Russian knapweed after cc with a variety of solvent systems. Pure dextrorotary material was crystallized from MeOH to give small needles melting at 155–157°. Slow crystallization from EtOH gave crystals suitable for X-ray analysis. The perspective view of the molecule is shown in Figure 1. The molecules are bonded in the crystal structure by a single hydrogen bond formed between O-20 and O-26 with a distance of 2.905 Å. Two intramolecular hydrogen distances are significant, viz., H-6 . . . H-9A = 2.272 Å and H-13A . . . O-24 = 2.869 Å. The proximity of H-6 to H-9A accounts for the nOe observed in the ^{1}H-nmr spectrum of repin. Repin [**5**] has the absolute configuration 1*R*,3*S*,4*S*,5*S*,6*S*,7*S*,8*S*,17*R*, the same as that of acroptilin [**4**] (10), which contains an epichlorohydrin moiety on the side chain. Formation of acroptilin from repin involves opening of the epoxide of repin to the epichlorohydrin with retention of configuration. These data would strongly suggest that the conversion is enzymatically controlled. On the other hand, the occurrence of subluteolide and repin as a 1:1 mixture in yellow starthistle (11) indicates that in this plant the reaction is not enzymatically controlled, or alternatively that enzymes for producing both epimers are present.

The detailed ^{1}H-nmr data for repin



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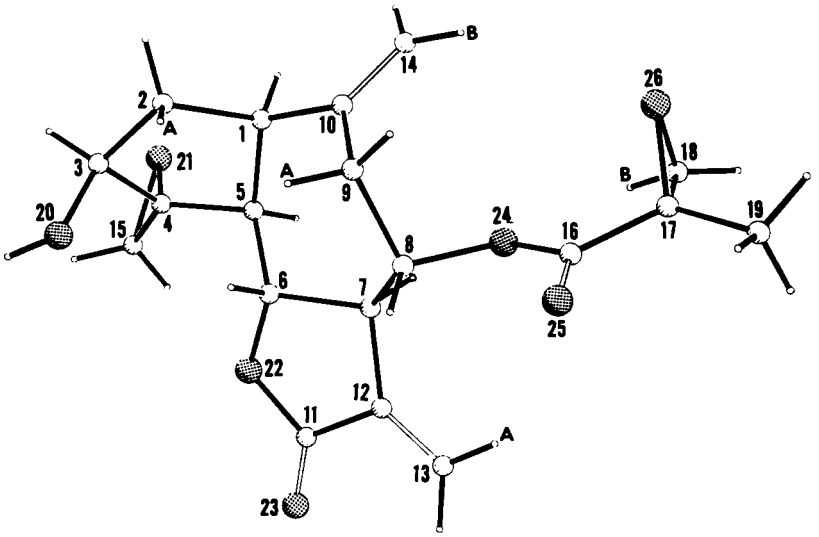

FIGURE 1. Perspective view of repin [**5**].

are given in the Experimental section. In addition to the expected coupling constants, a four-bond coupling was observed between H-1 and H-9B (0.5 Hz) as well as between H-9A and H-14A (1.3 Hz). The two coupling constants observed for H-13A and H-13B (3.5 and 3.1 Hz) are indicative not of geminal coupling but rather of four-bond coupling with H-7. H-18B is also coupled with the methyl group (C-19, $J<0.5$ Hz) which allowed assignment of the peak at δ 3.17 as H-18B. H-18B can assume the classical W form with the hydrogens on the methyl group because it is trans to the methyl group. H-18A cannot assume the W configuration, and hence no coupling is observed between H-18A and H-19.

Preliminary biological data obtained with repin and several other sesquiterpene lactones isolated from Russian knapweed and yellow starthistle (11) show significant activity toward chick embryo sensory neurons. In the presence of nerve growth factor (NGF), chick embryo neurons in vitro extend processes which are essential to the linking of neurons (12). However, in the presence of 80 nM repin these processes are 50% inhibited. The isomer, subluteolide, inhibits these same processes at 300 nM, whereas other sesquiterpenes of similar structure are much less active. It is clear that the configuration of the side chain has a significant effect on the toxicity of the molecule, i.e., the presence of a 17,18-epoxide in the *R* configuration is essential for maximum effect on the neural cells. The high toxicity of repin toward chick embryo sensory neurons suggests a causal relationship between this particular sesquiterpene lactone and the necrosis of neural cells in the substantia nigra of horses leading to ENE disease. A more detailed report on the biological effects of repin will be published elsewhere.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Purity of repin was monitored by tlc on 0.25 mm Si gel plates developed with EtOAc or $CHCl_3$-MeOH (9:1) and detected by spraying with 2% aqueous $KMnO_4$ solution. Nmr spectra were determined in $CHCl_3$ on a Nicolet NTC 200FT spectrometer at 200 MHz (^1H) and at 50.3 MHz (^{13}C) using TMS as an internal standard. Multiplicities for ^{13}C signals were determined by application of the carbon attached proton test (CAPT) sequence. Melting points are uncorrected.

EXTRACTION OF PLANT MATERIAL.—*A. repens* was collected during July 1988, near Discovery Bay, California. The dried aerial parts were ground in a hammer mill using a ¼-in. screen and extracted first with petroleum ether (bp 30–60°) then with CH_2Cl_2. The CH_2Cl_2 was evaporated, the residue was dissolved in 95% EtOH, and an equal volume of aqueous 4% $Pb(OAc)_2$ was added. After agitation for 1 h, the mixture was filtered to remove the precipitated chlorophyll and the filtrate evaporated at reduced pressure to remove the EtOH. The resulting aqueous mixture was then extracted with Et_2O and the ethereal extract chromatographed on Si gel with hexane/Me_2CO (gradient 5–30% Me_2CO). Repin crystallized from MeOH and was recrystallized from EtOH, mp 155–157°; ^1H-nmr (200 MHz, $CDCl_3$) δ 3.35 (H-1, dddd, $J=9.8$, 9.4, 8.2, 0.5), 1.82 (H-2A, ddd, $J=9.8$, 14.6, 4.3), 2.49 (H-2B, ddd, $J=9.4$, 14.6, 6.5), 3.99 (H-3, dd, $J=4.3$, 6.5), 2.06 (H-5, dd, $J=8.2$, 11.0), 4.63 (H-6, dd, $J=11.0$, 9.2), 3.07 (H-7, dddd, $J=9.2$, 9.3, 3.5, 3.1), 5.14 (H-8, ddd, $J=9.3$, 3.1, 5.0), 2.38 (H-9A, ddd, $J=3.1$, 14.9, 1.3), 2.73 (H-9B, dd, $J=5.0$, 14.9), 5.57 (H-13A, d, $J=3.5$), 6.21 (H-13B, d, $J=3.1$), 4.99 (H-14A, d, $J=1.3$), 5.20 (H-14B, br s), 3.33 (H-15A, d, $J=4.2$), 3.06 (H-15B, d, $J=4.2$), 2.83 (H-18A, d, $J=5.8$), 3.17 (H-18B, d, $J=5.8$), 1.62 (H-19, s); ^{13}C nmr (50.3 MHz, pyridine-d_5) δ 46.0 (C-1), 38.9 (C-2), 75.5 (C-3), 69.2 (C-4), 53.1 (C-5), 77.5 (C-6), 47.8 (C-7), 75.3 (C-8), 36.4 (C-9), 142.7 (C-10), 138.6 (C-11), 169.0 (C-12), 121.1 (C-13), 118.0 (C-14), 48.6 (C-15), 170.2 (C-16), 54.3 (C-17), 52.9 (C-18), 17.5 (C-19).

CRYSTAL DATA.[1]—Repin [**5**], $C_{19}H_{22}O_7$, $M=362.4$, monoclinic, space group $P2_1$, $a=9.624$ (3), $b=9.971$ (3), $c=10.454$ (5) Å, $\beta=114.90$ (3)°, $U=909.96$ Å^3, $D_c=1.32$

[1]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

$g \cdot cm^{-3}$, Z = 2, F(000) = 384, μ(CuKα) = 8.05 cm^{-1}. Final R = 0.062 (235 parameters), R_w = 0.060 for 1793 unique reflections with $|F_o| \geq 3\sigma|F_o|$ in the range $3° \leq 2\theta \leq 114°$, average parameter shift is $\pm 0.02\sigma$, and difference Fourier synthesis excursions are within ± 0.4 e·Å^{-3}.

DATA COLLECTION AND STRUCTURE REFINEMENT.—Intensity data were collected on a Nicolet R3 diffractometer with graphite monochromatized CuKα radiation (λ = 1.5418 Å) by the θ–2θ scan technique with variable scan speed (4–30°) at room temperature. The intensity data were corrected for background and Lorentz-polarization effects (13) but not for absorption or secondary extinction. The crystal structure was solved by direct methods. Atomic coordinates, thermal parameters, and scale factors were refined by a "blocked-cascade" full matrix least-squares procedure with the SHELXTL (14) program package. The function minimized was $\Sigma w(|F_o| - |F_c|)^2$, where $w = [\sigma^2|F_o| + 0.001\,|F_o|^2]^{-1}$. Scattering factors were from *International Tables for X-ray Crystallography* (15); those of oxygen were corrected for anomalous dispersion. Positions of all nonhydrogen atoms were refined anisotropically, and all hydrogen positions were estimated but verified in subsequent difference Fourier maps and included at invariant idealized values in the respective structure-factor calculation. The absolute configuration was determined by least-squares refinement of the parameters of both enantiomeric structures, giving a ratio of the two final R_w values of 1.014. According to Hamilton's statistical test (16), the enantiomer with the lower R_w value has a probability of being correct to a significance level better than 0.5%.

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