reaction mixture was cooled and filtered to yield 104 g. of crude product, m.p. 75–87°. Concentration of the mother liquor afforded another 10 g. of product. Development of the crude product on Silica Gel G plates with ether gave two spots, R_f 0.62 and 0.75. Recrystallization of the crude product from ethyl acetate–hexane five times gave only the less soluble isomer **15a**, m.p. 140–141°; R_f 0.62; ν_{max}^{KBr} 3390, 3300, and 3200 (NH), 2210 (CN), and 1680 cm.$^{-1}$ (amide CO).

Anal. Calcd. for $C_{12}H_{12}N_2O$: C, 72.00; H, 6.05; N, 14.00. Found: C, 71.97; H, 6.04; N, 14.30.

The soluble isomer **15b** was obtained by evaporating the mother liquor from the recrystallizations of isomer **15a** and placing 100 mg. of the residue on preparative Silica Gel G plates (1.5 mm. thick). Development with ether and extraction of the top band with acetone gave 50 mg. of isomer **15b**, m.p. 135.5–136°, R_f 0.75. The mixture melting point with isomer **15a** was 105–109°. The infrared spectrum in chloroform was identical with that of isomer **15a** in chloroform, but the spectrum of the KBr mull differed: ν_{max}^{KBr} 3410, 3350, 3300, and 3160 (NH), 2210 (CN), and 1680 cm.$^{-1}$ (amide CO).

Ring Closure of Isomer 15a.—One gram of isomer **15a** was dissolved in 5 ml. of concentrated sulfuric acid at 5° and maintained between 5–10° for 15 min. The green solution was poured over ice. No precipitation occurred until the solution was neutralized with 15% sodium hydroxide. The white solid (0.98 g., m.p. 175–190°) was collected by filtration and recrystallized from methanol to give 0.48 g. (48%) of **3a** (Z = $CONH_2$). A mixture melting point with a sample obtained by ring closure of **2b** was undepressed and their infrared spectra were identical.

Attempted Ring Closure of Isomer 15b.—A solution of 0.47 g. of isomer **15b** and 4 ml. of concentrated sulfuric acid was allowed to stand at room temperature for 2.5 hr. before pouring over ice. The precipitate was collected to give 0.27 g. of starting material, m.p. 115–120°. This was confirmed by identical infrared spectra and R_f values on t.l.c. Neutralization of the acidic mother liquor did not yield any precipitate.

A New Sesquiterpene Lactone with Analgesic Activity from *Helenium amarum* (Raf.) H. Rock

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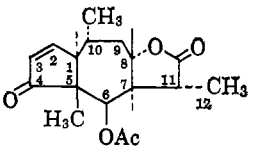
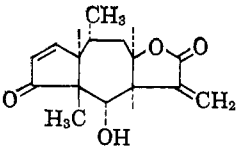
A new sesquiterpene lactone with potent analgesic activity has been isolated from *Helenium amarum* (Raf.) H. Rock. The structure of this substance has been shown to be Ia by correlation with another accompanying lactone (III), which in turn was related to desacetyldihydroisotenulin (XII). The activity of a number of related substances was investigated.

In connection with our general medicinal plant screening program, we had occasion to submit a crude extract of *Helenium amarum* (Raf.) H. Rock[1] to broad pharmacological investigation.[2] On subcutaneous administration, this material was found to have an analgesic activity in mouse tail flick test[3] which was not antagonized by nalorphine. It was also shown to inhibit the writhing syndrome in mice induced by the intraperitoneal injection of acetic acid. Further, the extract demonstrated no anticonvulsant properties nor did it cause any alteration in spinal reflexology in cats.

The above combination of biological properties seemed to warrant an attempt to identify the chemical individual responsible for this activity. The use of solvent distribution and of chromatographic techniques led to the isolation of two new substances. One, which we have called amaralin, $C_{15}H_{20}O_4$, m.p. 195–198°, $[\alpha]^{25}D$ +5°, was responsible for the major part of the analgesic action. The other, later shown to be identical with aromaticin,[4] $C_{15}H_{18}O_3$, m.p. 234–236°, $[\alpha]^{26}D$ +14°, was inactive. There also was a relatively large quantity of tenulin known to be the main sesquiterpene lactone of *Helenium amarum* (Raf.) H. Rock.[5] The empirical formulas of the two new substances indicated that they, too, were probably sesquiterpenes and, therefore, possibly related structurally to isotenulin or to helenalin.[6]



Isotenulin



Helenalin

That amaralin had one hydroxyl group could be inferred from its infrared absorption at 3430 cm.$^{-1}$ and by the formation of a monoacetate (Ib), which no longer had an infrared absorption in the hydroxyl region. This was further supported by the observed downfield shift of the n.m.r. signal at 3.81 p.p.m. in amaralin to 4.71 p.p.m. in the acetate (Table I). Such a shift would be expected for a proton attached to a carbon bearing a secondary alcohol.

Another band in the amaralin infrared spectrum at 1736 cm.$^{-1}$ could be attributed to a cyclic ketone or to a γ-lactone containing a conjugated exocyclic methylene, a grouping which is characteristic of many of these sesquiterpene lactones.[5] That this system was actually present in amaralin was further indicated by infrared bands at 1660 and 940 cm.$^{-1}$ (exocyclic double bond α

(1) The plant material was collected and identified by Harry E. Ahles, Botany Department, University of North Carolina, Chapel Hill, N. C., to whom our sincere thanks are due.

(2) We wish to express our appreciation to Dr. A. Plummer and his associates of our Macrobiology Division for the biological tests and for their kind permission to include some of their results in this paper.

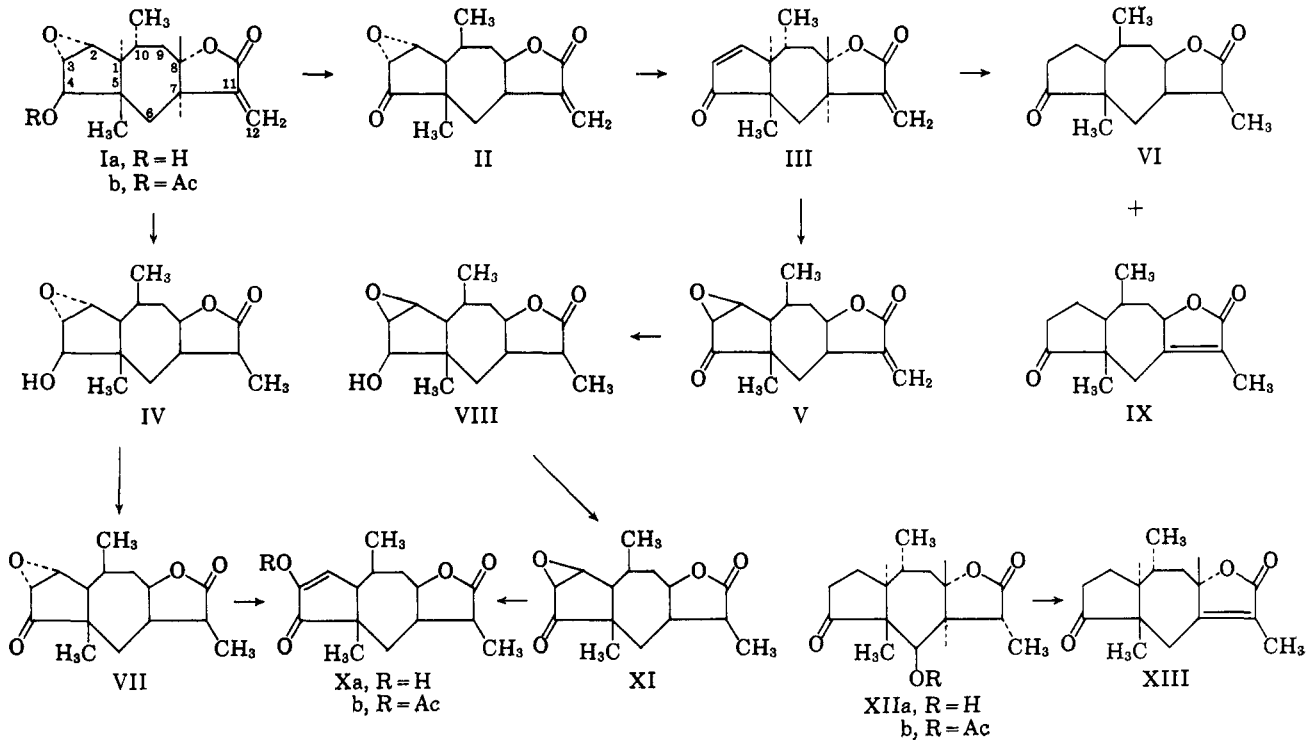
(3) L. Witkin, C. Huebner, F. Galdi, E. O'Keefe, P. Spitaletta, and A. Plummer, *J. Pharmacol. Exptl. Therap.*, **133**, 400 (1961).

(4) During the preparation of the manuscript of this paper, an article by J. Romo, P. Joseph-Nathan, and F. Diaz A. [*Chem. Ind.* (London), 1839 (1963)] appeared in which the isolation and structure of a new sesquiterpene lactone, aromaticin, was described. The physical properties of this substance corresponded with our second lactone and the methods of structure proof were exactly the same. A comparison sample of aromaticin kindly supplied by Dr. Romo through the courtesy of Dr. W. I. Taylor was shown to be identical with our substance by the usual criteria.

(5) For a review of the excellent and comprehensive work already done on the constituents of this and related species, see (a) W. Herz, A. Romo de Vivar, J. Romo, and N. Viswanathan, *J. Am. Chem. Soc.*, **85**, 19 (1963); (b) W. Herz, A. Romo de Vivar, J. Romo, and N. Viswanathan, *Tetrahedron*, **19**, 1359 (1963); and (c) W. Herz, *J. Org. Chem.*, **27**, 4043 (1962), and related papers.

(6) For simplicity in the development of our structural arguments we will assume this relationship, later to be demonstrated, and use the numbering system shown when referring to particular carbon and hydrogen atoms.

SCHEME I



to a carbonyl) and by the existence of the low-field doublets in the n.m.r. spectra at 6.17 and 5.46 p.p.m. which have been previously associated with this conjugated methylene.[5a] The presence of a broad single proton multiplet extending for 27 c.p.s. at approximately 4.3 p.p.m. was interpretable on the basis of a guaianolide skeleton as representing the proton attached to the carbon bearing the lactonized hydroxyl function. The complexity of this signal provided evidence which indicated that the lactone in amaralin was probably attached at C-8 as in isotenulin and helenalin, rather than at C-6 as is the case in some of the other natural sesquiterpene lactones. In that event the coupling of this proton would have resulted in a simple doublet.[7] Furthermore, this C-8 multiplet, ABXY type, can be analyzed as two overlapping quartets which seemed to imply that the stereochemistry at C-8 in amaralin was like that of the tenulin series (C-8 β-H), rather than like that of the helenalin series (C-8 α-H) where this signal is a triplet split again into doublets.[5a]

Hydrogenation of amaralin yielded a dihydro derivative (IV) which had only one infrared carbonyl band at 1770 cm.$^{-1}$ (unconjugated γ-lactone) and no longer had the band associated with the double bond. Nor did it exhibit the n.m.r. signals for the conjugated methylene, but did show a doublet signal in the methyl region at 1.20 p.p.m. indicative of a secondary methyl group.

A consideration of the above facts in the light of the wealth of information on the sesquiterpene lactones already provided by Herz and his collaborators[5] led to the postulation that dihydroamaralin was probably related to desacetyldihydroisotenulin (XIIa), but with some as yet unidentified difference in the fourth oxygen function. The presence of a ketone in amaralin seemed unlikely because of the lack of any appropriate absorption or of any reaction with the usual ketone reagents. In fact the nonreactivity of this fourth oxygen led us to consider some type of oxide bridge. Structures of this nature have been published[8] for some of the natural sesquiterpene lactones of the "normal" or guaianolide type (CH_3 at C-4), but so far as we are aware none belonging to the "abnormal" or pseudoguaianolide series (CH_3 at C-5) have been reported.

An insight into the further details of the structure of amaralin was had by the oxidation of its dihydro derivative (IV) with chromium trioxide to give ketone VII, whose infrared spectrum showed bands at 1765 (γ-lactone) and 1746 cm.$^{-1}$ (cyclopentanone).[9] Treatment of VII with base yielded a conjugated hydroxy ketone which exhibited a characteristic ultraviolet absorption spectrum: λ_{max} 261 mμ (ϵ 7000), λ_{max}^{KOH} 300 mμ (ϵ 6000), λ_{max}^{HCl} 259–261 mμ (ϵ 5600). These data seemed best explained on the basis of an enolic α-diketone structure such as Xa. Furthermore, reaction of VII with acetic anhydride and *p*-toluenesulfonic acid in acetic acid and subsequent work-up resulted in the isolation of two products, Xa and its acetate, Xb; the ultraviolet absorption spectrum of the latter showed a hypsochromic shift of 30 mμ expected for the acetate of an enolic conjugated ketone. These reactions can best be explained by assigning an α,β-epoxy ketone structure to VII.[10]

Assuming structure VII to be correct, then amaralin itself must contain an α-hydroxy epoxide, most prob-

(7) W. Herz, H. Watanabe, M. Miyazaki, and Y. Kishida, *J. Am. Chem. Soc.*, **84**, 2601 (1962).

(8) V. Procházka, Z. Čekan, and R. Bates, *Collection Czech. Chem. Commun.*, **28**, 1202 (1963); R. Bates, Z. Čekan, V. Procházka, and V. Herout, *Tetrahedron Letters*, **No. 17**, 1127 (1963); F. Sánchez-Viesca and J. Romo, *Tetrahedron*, **19**, 1285 (1963).

(9) A cycloheptanone could not be excluded. See D. Barton and P. de Mayo, *J. Chem. Soc.*, 142 (1956).

(10) For an analogy to the base-catalyzed reaction, see S. Winstein and R. Henderson, "Heterocyclic Compounds," Vol. 1, R. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., 1950, p. 52, and for one to the acid reaction, see H. House and R. Wasson, *J. Am. Chem. Soc.*, **79**, 1488 (1957).

TABLE I

N.M.R. SIGNALS[a]

	C-1 H	C-2 H[b] C-3 H	C-4 H[c]	C-5 CH_3	C-8 H	C-10 CH_3	C-11 CH_3	C-12 H[d]
Ia[c]		3.42 q ($H_{2,3}$ 3.7)	3.81 d (H_{OH} 5.3)	1.15	4.33 oq (H_7 *ca.* 11, H_9 *ca.* 9,3)	1.26 d (5.7)		6.17 d, 5.46 d (3.3)
Ib[h]		3.43 q ($H_{2,3}$ 3.7)	4.71	1.16	4.29 oq (H_7 *ca.* 9.5, H_9 *ca.* 9,3)	1.27 d (5.6)		6.14 d, 5.43 d (3.4)
II		3.65 q ($H_{2,3}$ 2.3)		1.18	4.31 oq (H_7 *ca.* 9, H_9 *ca.* 9,3)	1.28 d (5.4)		6.12 d, 5.44 d (3.3)
III[e]	2.80 ddd (H_2 2, H_3 3, H_{10} 10)	7.70 dd 6.17 dd (H_3 6, H_1 3) (H_2 6, H_1 2)		1.21	4.55 oq (H_7 9.6, H_9 8,3)	1.26 d (6.8)		6.21 d, 5.55 d (3.3)
IV[c]		3.35 q ($H_{2,2}$ 3.7)	3.70 d (H_{OH} 5.4)	1.11	4.25 oq (H_7 *ca.* 9, H_9 *ca.* 8.5,3)	1.20 d (5.7)	1.16 d (5.4)	
V[f]		3.86 q 3.35 d (H_3 2.6, H_1 1.2) (H_2 2.6)		1.24	4.32 oq (H_7 *ca.* 9, H_9 *ca.* 9,3.6)	1.29 d (6.6)		6.05 d, 5.73 d (3.3)
VI				1.04	4.31 oq (H_7 *ca.* 8.5, H_9 *ca.* 8,3)	1.20 d (6.7)	1.08 d (5.0)	
VII		3.64 q ($H_{2,3}$ 2.5)		1.19	4.34 oq (H_7 *ca.* 11, H_9 *ca.* 8,3.5)	1.28 d (5.1)	1.18 d (6.4)	
Xb	2.69 dd ($H_2$2, H_{10} 9.8)	7.20 d (H_1 2)		1.26	4.47 oq	1.20 d (7.3)	1.20 d (7.3)	
XIII[g]				1.00	5.02 d (H_9 *ca.* 11.8)	1.05 d (5.8)	1.73 q[g]	

[a] Spectra determined on a Varian A-60 spectrometer in deuteriochloroform. Values are given in p.p.m. relative to tetramethylsilane as internal standard. Numbers in parentheses denote coupling constants in c.p.s. of the hydrogen at top of column with the one indicated in parenthesis: H_2 means the hydrogen at C-2. Multiplicity of signals are designated as follows: d, doublet; q, quartet; oq, overlapping quartet. [b] Where a single signal is reported for C-2 H and C-3 H, it is the midpoint of a narrow quartet. [c] The signal of the hydroxyl hydrogen in Ia and IV is a doublet at 2.97 and 2.41 p.p.m., respectively, which in both cases becomes a singlet on the addition of deuterium oxide. [d] The signal for the vinyl proton oriented toward the carbonyl group of the lactone is at lower field. [e] We wish to thank Professor E. Wenkert for the decoupling studies on this compound and on certain helenalin derivatives. [f] The large chemical shift, 30 c.p.s., between hydrogens on C-2 and C-3 in the β-oxide was also observed for helenalin oxide. However, in the case of the compounds containing the α-oxide the shift was approximately 5 c.p.s. [g] The signal for the hydrogens at C-6 is split into an AB quartet centered at 2.63 p.p.m. (J = 20.4 c.p.s.). That for the hydrogens of the C-11 methyl group is split into a quartet by long-range coupling with C-8 H and with C-6 H, however, the constants for this (J = 1.5, 3 c.p.s.) have not been resolved. This long range coupling further results in a broadening of the C-8 H signal. [h] The signal of the acetyl methyl protons is at 2.17 p.p.m.

ably in the five-membered ring as shown in Ia. Additional evidence confirming this point was had by oxidizing amaralin to ketone II, which on reduction with chromous chloride yielded an α,β-unsaturated cyclopentenone identical with our second isolated product (III).

Certain possible structural features of III could be inferred from spectral data: ν 1768 (γ-lactone), 1707, 1590 (conjugated cyclopentenone), 1665, and 940 cm.$^{-1}$ (double bond); n.m.r. signals at 6.17 and 7.70 p.p.m. (α- and β-protons of cyclopentenone), 5.55 and 6.21 p.p.m. (conjugated exocyclic methylene); λ_{sh} 217 mμ (ε 14,000), combination of cyclopentenone and unsaturated lactone chromophores.[11] Catalytic reduction of III yielded a mixture from which a tetrahydro derivative (VI) could be isolated as the main product; ν 1760 (γ-lactone) and 1742 cm.$^{-1}$ (cyclopentanone); λ_{max} 218 mμ (ε 1070) (impurity, probably IX). The n.m.r. signals at 6.13 and 7.65 and at 5.52 and 6.18 p.p.m. were no longer present, but a new doublet appeared at 1.20 p.p.m. (C-11 CH_3). Epoxidation of III with alkaline peroxide formed an oxide (V) which was not identical with the corresponding substance (II) prepared from amaralin. However, treatment of its dihydro derivative (XI) with base did yield X which had already been derived from the corresponding amaralin derivative (VII) by a similar reaction. The patterns of the n.m.r. signals attributed to the C-8 protons in Ia, II, and V were practically identical and, hence, it is probable that no epimerization at this center took place during the transformation of Ia to II or of II to III or of III to V. But the nature of the reactions involved in converting II to III and VII to X is such that epimerization at C-1 cannot be excluded. However, in a reaction comparable to that involved in going from II to III, helenalin oxide[12] was reduced with chromous chloride to yield helenalin. Since no epimerization at C-1 apparently occurred in this case, it would seem unlikely that it did in the preparation of III from II. Hence II and V and, therefore, VII and XI must differ only in the configuration of the epoxide. These data were consistent with our postulated structure for III and a definite correlation with desacetyldihydroisotenulin was now necessary.

Having placed the oxygen functions in III at C-4 and C-8 it seemed to us that this correlation could best be accomplished by the removal of the C-6 oxygen function in desacetyldihydroisotenulin (XIIa). However, this was never accomplished in spite of numerous efforts to place appropriate groups in this substance at C-6 which might be removed by any of the well-known methods. We, therefore, resorted to the synthesis of a known compound which could be compared to IX. It has already been shown[5a] that in helenalin and similar substances, the exocyclic double bond of the lactone system undergoes isomerization during catalytic hydrogenation to yield an endocyclic conjugated lactone with a stable double bond at C-7–C-11. It has been previously mentioned that hydrogenation of III yielded, in addition to the tetrahydro derivative, another substance (IX) which when purified by chromatography had the ultraviolet and infrared absorption spectra

(11) G. Buchi and D. Rosenthal, *J. Am. Chem. Soc.* **78**, 3860 (1956).

(12) R. Adams and W. Herz, *ibid.*, **71**, 2551 (1949).

expected for such a system: λ_{max} 219 mμ (ε 16,000); ν 1755 (γ-lactone), 1739 (cyclopentanone), and 1668 cm.$^{-1}$ (conjugated double bond). Correlation of this substance and, therefore, III and amaralin with the tenulin series could be had by a comparison of its properties with those of anhydrodesacetyldihydroisotenulin (XIII) prepared by the literature method.[13] When this was done, it was found that IX was indeed identical with XIII. This identity confirmed the postulated skeletal structure for III and defined its stereochemistry as shown, because the C-7 hydrogen is considered to be α in all known cases.[5b] Since III has been related to amaralin, its structure and the stereochemistry of all of its asymmetric centers can now be assigned as shown in Ia, with the exception of those in the cyclopentane ring, and an inspection of the n.m.r. data permits a proposal for these.

Since the doublet at 3.81 p.p.m. (C-4 proton) collapsed to a singlet, 3.78 p.p.m., on the addition of D_2O, it must have been coupled to the hydroxyl proton and only minimally with C-3 H. Hence, the dihedral angle between C-3 H and C-4 H must approximate 90°. The two proton signal at 3.42 p.p.m., which is unaffected by the addition of D_2O, can now be assigned to the hydrogens on C-2 and C-3. The shape of this signal can best be described as a collapsed AB quartet, $J_{2,3}$ = 3.7 c.p.s., and, hence, not what would be expected if there were appreciable coupling between these hydrogens and either C-1 H or C-4 H. This is in agreement with the above stated relationship between C-3 H and C-4 H and further indicates that the dihedral angle between C-1 H and C-2 H must also approximate 90°.

An inspection of a Dreiding model of the various structures possible for this molecule showed that in only two were the above conditions all satisfactorily met. One corresponded to that shown in Ia and the other required that rings A and B be *cis* joined. Since we have already presented evidence that epimerization at C-1 was unlikely as a result of the reactions used in the correlation of amaralin with a substance of known *trans* structure, we prefer Ia as a representation of its stereochemistry, especially since this conforms in general with that already established for other members of this family of substances.

One of the reactions carried out early in our work in an attempt to gain knowledge about the nature of the fourth oxygen function in amaralin, led to a rather unexpected result. When this substance was treated with sodium borohydride, the product, formed in good yield, was found to be identical with dihydroamaralin (IV) produced by catalytic hydrogenation. In fact the metal hydride procedure was preferred since the product was free of the usual contaminating endocyclic unsaturated lactone isomerization by-product. So far as we are aware, this type of reduction of the exocyclic double bond of similarly unsaturated lactones has not been previously mentioned. However, a somewhat similar case has been reported for iresin.[14]

When this same reduction method was applied to helenalin acetate, we found that not only was the exocyclic methylene double bond reduced as in the case of amaralin, but also the α,β-unsaturated ketone was apparently reduced to a saturated alcohol mixture, which on reoxidation yielded the known tetrahydrohelenalin acetate with no spectral evidence for the regeneration of the conjugated ketone. The same result was obtained whether or not acid was used in the work-up.[15] Similar reductions of conjugated ketones are of course known in the steroids,[16] although the saturated alcohol is usually not the sole product.

Table II presents the biological activity of amaralin (Ia) and a number of related substances.

TABLE II

ANALGESIC ACTIVITY[a] OF SESQUITERPENE LACTONES

Compound	Dose mg./kg.[a]	Increase, %
Ia	10	64
	25	133
IV	100	92
III	100	None
V	100	28
Helenalin[b]	25	60
Helenalin oxide[c]	100	61
Tetrahydrohelenalin oxide[c]	100	None
Tenulin[d]	100	101
Isotenulin[d]	100	100
Isotenulin oxide[e]	100	64
XIIb[d]	100	51

[a] Drugs administered subcutaneously. [b] See ref. 5a. Material kindly supplied by Dr. W. I. Taylor. [c] See ref. 12. [d] See ref. 13. [e] See Experimental.

Experimental

All melting points are uncorrected. Ultraviolet spectra were determined in 95% ethanol unless otherwise indicated on a Beckman DK-1 spectrophotometer; infrared spectra were run in Nujol on a Perkin-Elmer Model 21 spectrophotometer. Rotations were measured in chloroform solution unless stated otherwise.

Isolation of Amaralin (Ia).—A 3.2-kg. sample of finely ground leaves and stems of *Helenium amarum* (Raf.) H. Rock was extracted twice with 12 l. of boiling methanol and once with 12 l. of hot water. The combined extract was evaporated *in vacuo* to a thick paste and partitioned in countercurrent fashion between 1-butanol and water, using portions of 1, 0.5, and 0.5 l. each. Evaporation of the 1-butanol fraction *in vacuo* yielded 236 g. of crude extract.

A 100-g. portion of the above was finely powdered and digested with 400 ml. of boiling benzene for 2 hr. After filtration of the hot solution to remove 27 g. of insoluble material, the filtrate was concentrated to a volume of 250 ml. and allowed to crystallize at room temperature overnight, yielding 17 g. of crude crystalline tenulin. The benzene filtrate was chromatographed on 1350 g. of Woelm grade II–III neutral alumina. The column was eluted with 10 l. of benzene, 9 l. of benzene–CH_2Cl_2 (50%), and 16 l. of CH_2Cl_2. The final 10 l. of CH_2Cl_2 eluate yielded 1.14 g. of amaralin,[17] m.p. 195–198° after crystallization from benzene; $[\alpha]^{25}D$ +5°; ν 3430 (hydroxyl), 1736 (γ-lactone), 1660, and 940 cm.$^{-1}$ (double bond).

Anal. Calcd. for $C_{15}H_{20}O_4$; C, 68.16; H, 7.63. Found: C, 68.48; H, 7.40; mol. wt. (mass spectroscopy), 264.

Isolation of III.—The benzene eluates from a number of chromatograms used for the isolation of Ia were combined, evaporated to a pasty solid (35 g.) and dissolved by refluxing in 50 ml. of cyclohexane. The solution was allowed to crystallize overnight at 0–5°, yielding crude III. Recrystallization from cyclohexane and from ethyl acetate–CH_2Cl_2 yielded 2 g. of material, m.p. 234–236°; $[\alpha]^{26}D$ +14°; ν 1768 (γ-lactone), 1707, 1590 (cyclopentenone), 1655, and 940 cm.$^{-1}$ (double bond).

(13) W. Herz, W. Rohde, K. Rabindran, P. Jayaraman, and V. Viswanathan, *J. Am. Chem. Soc.*, **84**, 3857 (1962).

(14) C. Djerassi and W. Rittel, *J. Am. Chem. Soc.* **79**, 3528 (1957).

(15) J. Marshall and W. Johnson, *J. Org. Chem.*, **28**, 595 (1963).

(16) F. Sondheimer, M. Velasco, E. Batres, and G. Rosenkranz, *Chem. Ind.* (London), 1482 (1954); D. Kupfer, *Tetrahedron*, **15**, 193 (1961).

(17) A recent repetition of this isolation procedure using plant material collected a year later has so far not yielded any amaralin.

Anal. Calcd. for $C_{15}H_{18}O_3$: C, 73.14; H, 7.37. Found: C, 73.16; H, 7.53.

The melting point of a mixture of III and aromaticin[4] showed no depression and the infrared spectra of the two substances were identical.

Amaralin Acetate (Ib).—Ia (100 mg.) was acetylated with pyridine (1 ml.) and acetic anhydride (1 ml.) at room temperature. The reaction mixture was poured into ice–water (10 ml.), made basic with 2 ml. of aqueous ammonia, and extracted with CH_2Cl_2. After washing and drying, the extract was evaporated and the residue was warmed with ether yielding 50 mg. of crude crystalline acetate. Recrystallization from cyclohexane–CH_2Cl_2 afforded 20 mg. of Ib, m.p. 140–142°; $[\alpha]^{26}D$ −16°; ν 1761 (γ-lactone), 1741, 1245 (acetate) 1662, and 940 cm.$^{-1}$ (double bond).

Anal. Calcd. for $C_{17}H_{22}O_5$: C, 66.65; H, 7.24. Found: C, 66.84; H, 7.38.

Dihydroamaralin (IV). A. Catalytic Reduction.—A solution of 200 mg. of Ia in 30 ml. of methanol was hydrogenated over 200 mg. of 10% palladium on carbon (presaturated with hydrogen). Approximately 1 molar equiv. of hydrogen was absorbed during 2 hr. Filtration of catalyst and evaporation *in vacuo*, followed by crystallization of the residue from ethyl acetate yielded 80 mg. of IV, m.p. 218–219°, ν 1770 cm.$^{-1}$.

B. Sodium Borohydride Reduction.—Ia (200 mg.) in methanol (8 ml.) was treated with 200 mg. of sodium borohydride in 4 ml. of methanol at room temperature. After 1 hr. the mixture was diluted with water, acidified with hydrochloric acid, and extracted four times with 25-ml. portions of CH_2Cl_2. The combined extracts were dried and evaporated *in vacuo*. Crystallization of the residue from CH_2Cl_2–ethyl acetate yielded 135 mg. of IV, m.p. 223–225°, $[\alpha]^{26}D$ +23°, ν 3446 (hydroxyl) and 1770 cm.$^{-1}$ (γ-lactone).

Anal. Calcd. for $C_{15}H_{22}O_4$: C, 67.64; H, 8.33. Found: C, 67.08; H, 8.29.

Oxidation of IV to VII.—A solution of 100 mg. of IV in 4 ml. of acetone was cooled to 0°, stirred vigorously, and treated with 5 drops of 8 *N* chromic acid[18] which imparted a persistent orange color to the solution. After 1 min. the mixture was diluted with 40 ml. of water and extracted with three 20-ml. portions of CH_2Cl_2. The solvent after drying was evaporated *in vacuo* and the residue was crystallized from ethyl acetate–CH_2Cl_2 yielding 77 mg. of VII, m.p. 238–240°, $[\alpha]^{26}D$ +116°, ν 1765 (γ-lactone) and 1746 cm.$^{-1}$ (cyclopentanone).

Anal. Calcd. for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 68.70; H, 7.59.

Preparation of Xa.—A suspension of 100 mg. of VII in 2.7 ml. of 5% sodium hydroxide was heated on the steam bath for 0.5 hr. effecting gradual solution. It was cooled, acidified with 2 *N* hydrochloric acid, and after a short time yielded 65 mg. of needles which on crystallization from ethyl acetate–CH_2Cl_2 had m.p. 245–246°; $[\alpha]^{26}D$ +89°; λ_{max} 261 mμ (ϵ 7000), λ_{max}^{KOH} 300 mμ (ϵ 6000), λ_{max}^{HCl} 259–261 mμ (ϵ 5600); ν 3345 (hydroxyl), 1750 (γ-lactone), 1700 (ketone), and 1650 cm.$^{-1}$ (double bond).

Anal. Calcd. for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 67.37; H, 7.77.

Reaction of VII with Acetic Anhydride, *p*-Toluenesulfonic Acid.—A mixture of 60 mg. of VII, 150 mg. of *p*-toluenesulfonic acid, 1.2 ml. of acetic anhydride, and 2.4 ml. of acetic acid was heated at 60–65° for 3 hr. and then allowed to stand overnight at room temperature. At the end of this time it was partially evaporated *in vacuo*, diluted with 20 ml. of water, and extracted several times with CH_2Cl_2. The combined, water-washed CH_2Cl_2 extracts were evaporated *in vacuo*, leaving an oily product which could not be crystallized. It was chromatographed in benzene solution on 3 g. of Woelm activity II–III neutral alumina. Benzene eluted 36 mg. of solid which crystallized from hexane–CH_2Cl_2 as shiny needles of Xb, 26 mg., m.p. 191–192°; λ_{max} 230–232 mμ (ϵ 8700) and 315 mμ (ϵ 60), λ_{min} 280 mμ (ϵ 30), λ_{max}^{KOH} 298–300 mμ (ϵ 6300), λ_{max}^{HCl} 244–248 mμ; ν_{CHCl_3} 1764 (γ-lactone and enol acetate) and 1719 cm.$^{-1}$ (unsaturated ketone).

Anal. Calcd. for $C_{17}H_{22}O_5$: C, 66.65; H, 7.24. Found: C, 66.01; H, 7.23.

The above column was then eluted with increasing amounts of CH_2Cl_2 and finally CH_2Cl_2 and 4% methanol yielded 17 mg. of a second substance which after crystallization from CH_2Cl_2–hexane weighed 12 mg., m.p. 235–238°. This substance was shown to be identical with Xa previously prepared.

Anal. Calcd. for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 68.21; H, 7.51.

Oxidation of Amaralin.—A solution of 52 mg. of Ia in 2 ml. of acetone was cooled to 5° and oxidized with the chromic acid mixture as described for the preparation of VII. The experiment yielded 35 mg. of II, m.p. 244–245° after crystallization from methanol–CH_2Cl_2; $[\alpha]^{26}D$ +101°; ν 1757 (γ-lactone), 1739 (cyclopentanone), and 1660 sh cm.$^{-1}$ (double bond).

Anal. Calcd. for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92. Found: C, 68.68; H, 6.86.

Reduction of II to III with Chromous Chloride.—A mixture of II (21 mg.) in 1 ml. of acetic acid was warmed slightly to effect solution, cooled to room temperature, and treated with 0.5 ml. of 0.4 *N* chromous chloride solution. After 0.5 hr. the mixture was diluted with water and the product was extracted with CH_2Cl_2. Chromatography of the 19 mg. of crude substance on 700 mg. of Woelm II–III neutral alumina afforded from the benzene eluate 5 mg. of solid. This was recrystallized from cyclohexane–CH_2Cl_2, yielding 1.37 mg. of crystals, m.p. 225–228°. A comparison of the infrared spectrum of this substance with that of isolated III demonstrated their identity, and the melting point of a mixture showed no depression.

Reduction of III.—A mixture of 800 mg. of III and 800 mg. of 10% palladium on carbon in 160 ml. of ethyl acetate was hydrogenated at atmospheric pressure and room temperature with very slow and interrupted periods of stirring. During the course of 4 hr., 160 ml. of hydrogen was consumed. The product was worked up in the usual way to obtain a mixture of VI and IX. Repeated chromatography on Woelm II–III and III–IV neutral alumina afforded from the cyclohexane–benzene (2:1) eluates 65 mg. of VI, m.p. 135–136°; λ_{max} 218 mμ (ϵ 1070), indicates some contamination with IX; ν 1760 (γ-lactone) and 1742 cm.$^{-1}$ (cyclopentanone).

Anal. Calcd. for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 72.29; H, 8.83.

The slower moving IX was eluted with 1:1 cyclohexane–benzene. Even after repeated chromatography the latter compound was slightly contaminated with VI. The purest sample of IX had m.p. 140–143°, $[\alpha]^{26}D$ +164° (EtOH), λ_{max} 219 mμ (ϵ 16,000). This substance was later shown to be identical with anhydrodesacetyldihydroisotenulin (XIII) by the usual criteria.

Epoxidation of III.—A solution of 200 mg. of III in 5 ml. of CH_2Cl_2 plus 5 ml. of methanol was cooled to 0° and allowed to react with a cold mixture of 0.5 ml. of 30% hydrogen peroxide, 0.5 ml. of 4 *N* sodium hydroxide, and 0.5 ml. of water. After stirring for 20 min. at 0° the mixture was poured into 20 ml. of water and acidified with dilute hydrochloric acid. It was extracted with CH_2Cl_2. Work-up in the usual way and crystallization from CH_2Cl_2–methanol yielded 160 mg. of V, m.p. 188–189°; $[\alpha]^{26}D$ +29°; ν 1766 (γ-lactone), 1741 (cyclopentanone), and 1666 cm.$^{-1}$ (double bond).

Anal. Calcd. for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92. Found: C, 68.76; H, 6.88.

Reduction of V.—A slurry of 70 mg. of V in 4 ml. of methanol was treated at 0° with 70 mg. of sodium borohydride in 2 ml. of methanol. At the end of 1 hr. the solution was diluted with 20 ml. of ice–water and acidified with hydrochloric acid. Extraction with CH_2Cl_2 in the usual manner yielded crystalline VIII, m.p. 180–195°, ν 3500 (hydroxyl) and 1743 cm.$^{-1}$ (γ-lactone).

Oxidation of VIII.—The above compound (70 mg.) in 3 ml. of acetone was oxidized with chromic acid in the way described for the preparation of VII. There was obtained 60 mg. of XI, m.p. 158–160° after recrystallization from CH_2Cl_2–ether, ν 1772 (γ-lactone) and 1741 cm.$^{-1}$ (cyclopentanone).

Base Treatment of XI.—A 35-mg. sample of XI was treated with sodium hydroxide and worked up as described for the preparation of X from VII, yielding 30 mg. of needles, m.p. 245–246° after recrystallization from ethyl acetate–CH_2Cl_2. A mixture melting point with the basic rearrangement product (X) obtained from VII exhibited no depression and the infrared and ultraviolet absorption curves were practically superimposable.

Isolation of Tenulin.—The 17 g. of crude tenulin obtained from benzene crystallization during the isolation of amaralin was purified by filtration in CH_2Cl_2 through a short column of Woelm activity II–III neutral alumina. Crystallization from ethyl acetate–petroleum ether gave 12 g. of tenulin, m.p. 214–216°, lit.[9] m.p. 215°; $[\alpha]^{26}D$ −22°; λ_{max} 226 mμ (ϵ 8500); ν 3407 (hydroxyl), 1772 (γ-lactone), and 1713 cm.$^{-1}$ (cyclopentenone).

(18) A. Bowers, T. Halsall, E. Jones, and A. Lemin, *J. Chem. Soc.*, 2555 (1953).

Anal. Calcd. for $C_{17}H_{22}O_5$: C, 66.65; H, 7.24. Found: C, 66.47; H, 7.20.

Isotenulin.—The method of Barton[8] was followed using tap water to convert 2 g. of tenulin to isotenulin, yielding 1.8 g., m.p. 161–163°, lit.[9] m.p. 160–161°; $[\alpha]^{26}D$ +9° (EtOH); λ_{max} 225 mμ (ε 8800); ν 1775 (γ-lactone), 1705 and 1590 (cyclopentenone), 1750, and 1230 cm.$^{-1}$ (acetate).

Anal. Calcd. for $C_{17}H_{22}O_5$: C, 66.65; H, 7.24. Found: C, 66.52; H, 7.14.

Dihydroisotenulin.—Isotenulin (2.1 g.) was reduced catalytically over 200 mg. of 10% palladium on carbon in methanol yielding 1.9 g. of dihydroisotenulin, m.p. 147–149° crystallized from cyclohexane–CH_2Cl_2 (lit.[9] m.p. 148–149°.

Anhydrodesacetyldihydroisotenulin.—Dihydroisotenulin (2 g.) was converted[13] through the intermediates desacetyldihydroisotenulin and its methanesulfonyl ester to anhydrodesacetyldihydroisotenulin (0.75 g.), m.p. 130–132°. Further crystallization from ether, filtration in benzene through Woelm activity II–III neutral alumina, and crystallization from hexane–CH_2Cl_2 raised the melting point to 143–144°, lit.[13] m.p. 143.5–144°; $[\alpha]^{26}D$ +170° (EtOH); λ_{max} 218–220 mμ (ε 14,800); ν 1755 (γ-lactone), 1739 (cyclopentanone), and 1668 cm.$^{-1}$ (double bond).

Anal. Calcd. for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.51; H. 8.08.

Isotenulin Oxide.—To 500 mg. of isotenulin in 12 ml. of methanol, cooled to −7°, was added a mixture of 0.5 ml. of 30% hydrogen peroxide, 0.5 ml. of water, and 106 mg. of sodium carbonate. The mixture was stirred for 10 min. before the addition of 20 ml. of water to complete precipitation of the solid. After filtering and drying, it weighed 400 mg. and melted indistinctly at 85–95°. It resisted all attempts at recrystallization and had ν 1750–1780, sh 1715 cm.$^{-1}$; λ_{max} 225 mμ (ε 1950).

Anal. Calcd. for $C_{17}H_{22}O_6$: C, 63.34; H, 6.88. Found: C, 63.52; H, 6.82.

Reduction of Helenalin Oxide to Helenalin with Chromous Chloride.—An 18-mg. sample of helenalin oxide[12] was reduced with 0.4 *N* chromous chloride solution in exactly the same manner as described for the reduction of II. The crude reaction product crystallized from the CH_2Cl_2 extract and after recrystallization from CH_2Cl_2 afforded 10 mg. of substance, m.p. 164–166°, which was shown to be identical with a known sample of helenalin by the usual criteria.

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Benzene Extractives of Lodgepole Pine Bark. Isolation of New Diterpenes[1]

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Lodgepole pine bark is unique in that over one-fourth is soluble in benzene. The benzene extract has been fractionated and the neutral materials extensively investigated. These consist of esterified wax and fatty acids, homologous wax alcohols, *n*-paraffins, sterols, and diterpenes. The most significant discovery is that 21% of the benzene extract consists of the new diterpene alcohol, 13-epimanool (**1**) ($\Delta^{8(20),14}$-labdadien-13α-ol). Three other new diterpenes with the labdane skeleton, hydroxyepimanool (**2**), contortolal (**3**), and contortadiol (**4**), were also isolated.

Pinus contorta Dougl., commonly known as lodgepole pine, is an important commercial western tree and one of the oldest species on the North American continent.[3] It grows from Alaska and the Yukon Territory to Baja California, Mexico, and from the Pacific Coast to the eastern edge of the Rocky Mountains. Some botanists have claimed there are a number of varieties of lodgepole pine, especially the inland form, which shows some difference from the coastal form.[4] The "Checklist of Native and Naturalized Trees of the United States (Including Alaska)," however, lists all varieties as *P. contorta*.[5]

Lodgepole pine has a thin brown bark with many loose scales. Hergert has investigated this bark[6] and found it to be a rich source of flavonoids, especially myricetin, whose isolation from this bark has been patented.[7] Quercetin, dihydroquercetin, dihydromyricetin, aromadendrin, and pinobanksin were also found in lesser amounts. A low yield of a good tannin has also been obtained from this bark.[8]

The turpentine of lodgepole pine has been investigated and found to consist almost entirely of *l*-β-phellandrene with small amounts of *l*-α-pinene sometimes present.[4] Hergert has also identified small amounts of β-pinene, Δ^3-carene, and β-camphene in the turpentine by gas chromatography.[9] Schorger reported that the oleoresin contains abietadienic acids, and that the needle and twig oil contains *l*-α-pinene, *l*-β-pinene, *l*-phellandrene, camphene, dipentene, *l*-borneol, bornyl acetate, methylchavicol (?), cadinene, and a trace of furfural.[10]

During a study of the chemical composition of common North American pulpwood barks,[11] it was found

(1) Presented at the 144th National Meeting of the American Chemical Society, Los Angeles, Calif., April, 1963, p. 10D of abstracts. Part of these results are included in the undergraduate thesis of J. H. S. at the University of Wisconsin. Previous paper in this series: W. C. Nickles and J. W. Rowe, *Forest Prod. J.*, **12**, 374 (1962).

(2) Maintained at Madison, Wis., in cooperation with the University of Wisconsin.

(3) D. A. Zischke, "Lodgepole Pine," U. S. Forest Products Laboratory Rept. No. 2052, 1956.

(4) N. T. Mirov, "Composition of Gum Turpentines of Pines," U.S.D.A. Tech. Bull. No. 1239, U. S. Government Printing Office, Washington, D. C., 1961, p. 117, and references cited therein.

(5) E. L. Little, Jr., U.S.D.A. Handbook No. 41, U. S. Government Printing Office, Washington, D. C., 1953, p. 263.

(6) H. L. Hergert, *J. Org. Chem.*, **21**, 534 (1956).

(7) H. L. Hergert, U. S. Patent 2,870,165 (Jan. 20, 1959); Canadian Patent 612,236 (Jan. 10, 1961).

(8) Oregon State Board of Forestry, Biennial Report of State Forester to Governor, 1948/49–1949/50, 1950, p. 65.

(9) H. L. Hergert, private communication.

(10) A. W. Schorger, *J. Ind. Eng. Chem.*, **7**, 24 (1915).

(11) Y.-P. Chang and R. L. Mitchell, *Tappi*, **38**, 315 (1955).