

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: June 18, 1976

Project Title: *The Anti-tumor Agent of Aplopappus Heterophyllus*

Project No: *G-33-C01*

Project Director: *Dr. L. H. Zalkow*

Sponsor: *DHEW/PHS/NIH - National Cancer Institute, Bethesda, Maryland*

Agreement Period: From 6/30/76 Until 6/29/77*
**01 year; overall grant period 6/30/76 - 6/29/79*

Type Agreement: *Grant No. 1 R01 CA18819-01*

Amount: *\$62,710 PHS funds*
8,355 GIT (G-33-382)
\$71,065 Total

Reports Required: *Interim Progress Report*
Terminal Progress Report

Sponsor Contact Person (s):

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Program Director for Drug Development
Division of Cancer Research Resources
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Contractual Matters

(thru OCA)

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DHEW, PHS, NIH
Bethesda, Maryland 20014
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Defense Priority Rating:

Assigned to: *Chemistry* (School/Laboratory)

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Project Code (GTRI)
Other _____

10-20
B-158

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT TERMINATION

Date: August 15, 1977

Project Title: *The Anti-Tumor Agent of Aplopappus Heterophyllus*

Project No: *G-33-C01*

Project Director: *Dr. L. H. Zalkow*

Sponsor: *DHEW/PHS/NIH - National Cancer Institute, Bethesda, MD*

Effective Termination Date: 6/30/77 (End 01 year)

Clearance of Accounting Charges: by 6/30/77

Grant/Contract Closeout Actions Remaining:

- ☐ Final Invoice and Closing Documents
- ☐ Final Fiscal Report
- ☐ Final Report of Inventions
- ☐ Govt. Property Inventory & Related Certificate
- ☐ Classified Material Certificate
- ☐ Other Annual Report of Expenditures due by 9/30/77.

NOTE: FOLLOW-ON PROJECT (02 YEAR) IS G-33-C02.

Assigned to: Chemistry (School/Laboratory)

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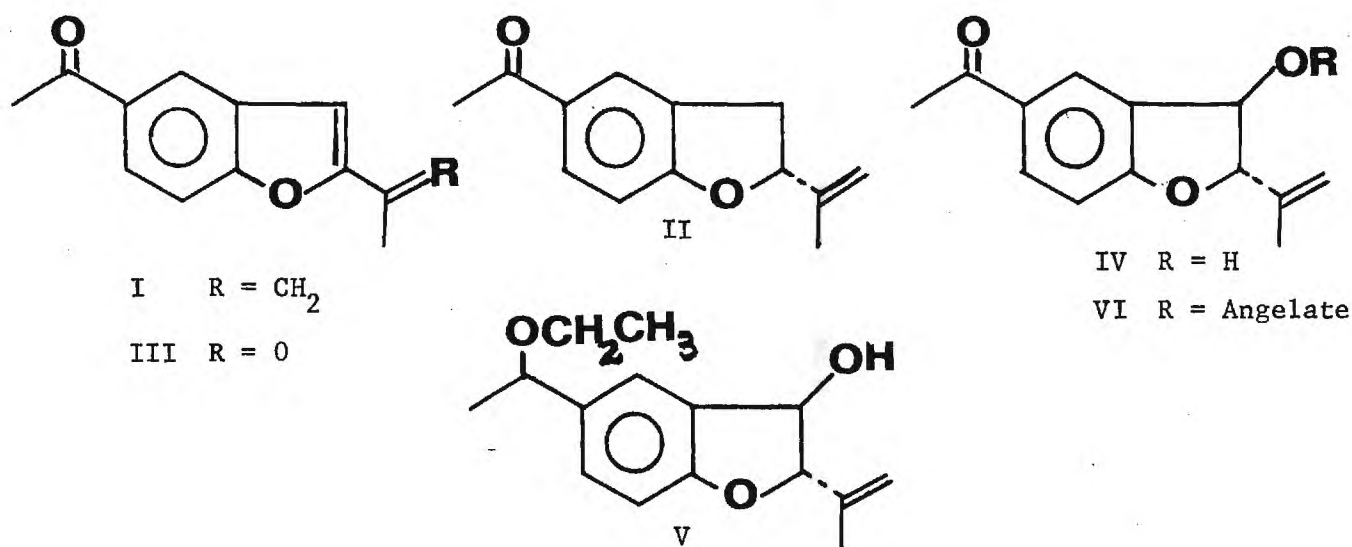
APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1 →		GRANT NUMBER	
SECTION IV—SUMMARY PROGRESS REPORT		CA-18819-02	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial)		PERIOD COVERED BY THIS REPORT	
Zalkow, Leon H.		FROM	THROUGH
NAME OF ORGANIZATION		July 1, 1976	June 30, 1977
Georgia Institute of Technology			
TITLE (Repeat title shown in Item 1 on first page)			
"The Anti-tumor Agent of <u>Aplopappus heterophyllus</u> "			

1. List publications: (a) published and not previously reported; (b) in press. Provide five reprints if not previously submitted.
2. List all additions and deletions in professional personnel and any changes in effort.
3. Progress Report. (See Instructions)

1. Publications: (a) L. H. Zalkow, R. N. Harris, III, D. Van Derveer, and J. A. Bertrand, "Isocomene. A Novel Sesquiterpene from Isocoma Wrightii, X-ray Crystal Structure of the Corresponding Diol," J.C.S. Chem. Commun., Accepted; (b) L. H. Zalkow, B. A. Ekpo and N. I. Burke, "Triterpenes of Isocoma Wrightii (Haplopappus heterophyllus)," Phytochemistry, accepted; (c) L. H. Zalkow and R. N. Harris, III, "1,2,3,4-Tetrahydro-1,1,5,6-tetramethylnaphthalene. An Unusual Natural Product from Isocoma Wrightii," Tetrahedron Letters, submitted; (d) L. H. Zalkow, L. Gelbaum, M. Ghosal and T. J. Fleischmann, "The Cooccurrence of Desmethylenecalin and Hydroxytremetone in Eupatorium Rugosum (E. urticaefolium)," Phytochemistry, accepted; (e) L. H. Zalkow, B. Ekpo, R. Harris, III and J. Novak, "The Benzofurans of Isocoma Wrightii (Haplopappus heterophyllus)," in preparation.
2. Messrs. J. R. Novak, Jr., and James T. Baxter, graduate students, and Messrs. T. J. Fleischmann and M. D. Witcher, undergraduates, who worked on this project during the 1976-1977 academic year will not continue during the next fiscal year.

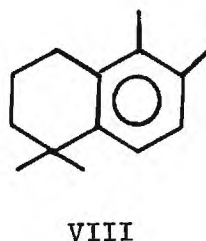
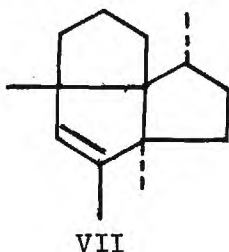
3. Progress Report

During the period 7/1/76-4/25/77 we have concentrated on developing isolation procedures for the various chemical constituents of *Isocoma Wrightii* with good success, having isolated, besides the previously reported compounds, a number of new and novel compounds, several of whose structures have already been elucidated. Thus, we have found that by utilizing the revised NCI fractionation procedure (2/22/77), we were able to isolate, in good yield, the following benzofurans from the methanol-water (9:1) fraction after base washing: dehydrotremetone (I), tremetone (II), 2,5-diacetylbenzofuran (III), toxol (IV) and the new derivative V. The latter structure, including stereochemical assignments remains to be conclusively verified.



From the hexane fraction the new benzofuran, toxyl angelate (VI) was isolated. The 1H and ^{13}C NMR and mass spectra of each of these benzofurans has been measured and tabulated providing excellent criteria for further structure elucidations of the remaining benzofurans detected by g.l.c. and preliminary spectral comparisons. These results will appear shortly in the Ph.D. dissertation of Mr. John Novak and in a manuscript under preparation. We have submitted samples of 2,5-diacetylbenzofuran (III) (NSC 247532), tremetone (II) (247531), toxol (IV) (247530) and toxyl angelate (VI) (282185) for screening by NCI, but none of these have shown sufficient activity to be of particular interest. However, we have noticed that the T/C's increase in the order, toxyl angelate > toxol > 2,5-diacetylbenzofuran suggesting to us that the active substituent may, in fact, be a member of this class of compounds.

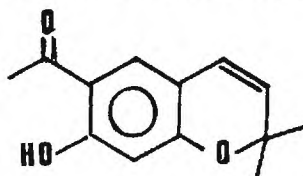
From the steam volatile plant extracts two new and novel compounds, isocomene (VII) and 1,2,3,4-tetrahydro-1,1,5,6-tetramethylnaphthalene (VIII) have been isolated and their structures elucidated (see attached preprints). A substance which appears



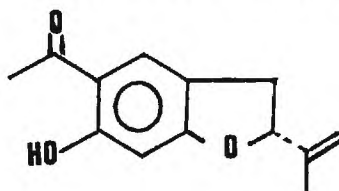
to be isomeric with isocomene has not yet been fully characterized and from the hexane extract an uncharacterized sesquiterpenoid furan has been isolated. Besides a number of common monoterpenes the sesquiterpenes caryophyllene and caryophyllene oxide were also isolated. The two triterpenes friedelin and friedelan-3 α -ol were isolated from the hexane extract (see attached preprint). The hexane extract has likewise been shown to contain the C₂₅, C₂₇, C₂₉, C₃₁ and C₃₃ straight chain hydrocarbons as the principal hydrocarbons with only traces of the even-numbered isomers. The water soluble extract has been shown to contain alkaloids which give a positive Mattocks test for pyrrolizidine N-oxides.

From the saponified non-ketone fraction of the ethanolic extract, the steroids stigmasta-8(14), 22-dien-3 β -ol, stigmast-8(14)-en-3 β -ol and stigmasta-5,22-dien-3 β -ol were isolated and characterized. In addition this fraction gave phytol and squalene.

Since Eupatorium rugosum has been reported to show a clinically identical disease in higher animals to that shown by Isocoma Wrightii, a phytochemical investigation of this plant was undertaken. It has been shown to contain desmethylenecalalin (IX) and hydroxytremetone (X) (see attached preprint). In



IX



X

addition, stigmasterol and the palmitic acid ester of taraxasterol were isolated from this plant.

The undersigned agrees to accept responsibility for the scientific and technical conduct of the project and for provision of required progress reports if a grant is awarded as the result of this application.

April 27, 1977

Date

Principal Investigator or
Program Director

L. H. Zalkow, B. A. Ekpo, N. I. Burke

School of Chemistry, Georgia Institute of Technology

Atlanta, Georgia 30332

(Received , Accepted)

Key Word Index - Isocoma Wrightii (Haplopappus heterophyllus); Compositae; friedelin, friedelan-3 α -ol.

Plant: Isocoma Wrightii (Haplopappus heterophyllus). Source: general area of Roswell, New Mexico. Previous Work: Isolation of benzofurans¹⁻⁴, steroids^{5,6}, mono⁷ and sesquiterpenes^{7,8} and fatty acids⁷.

Present Work: The entire dried, above ground plant, after grinding, was continuously extracted with hexane. Steam distillation of the hexane extract (400 g) gave 385 g of non-volatile residue which yielded 360 g ether soluble material. The latter was partitioned with benzene: ethanol: water (3:0.75:0.25) to give a benzene rich fraction, which after washing with ice cold 5% sodium hydroxide yielded 90 g of neutral material. Chromatography of 20 g of this neutral fraction of 1 kg of activity II Merck neutral alumina gave 6.3 g in the hexane eluent, 3.8 g in the 1:1 hexane benzene eluent, 5.3 g in the 1:4 hexane-benzene eluent, 1.0 g in the benzene eluent, 1.7 g in the chloroform eluent and 1.5 g in the methanol strip.

From the early fractions of the 1:1 hexane-benzene eluent a white solid was deposited on evapoartion of the solvent. Recrystallization from hexane gave friedelin as white needles, single peak by g.l.c. on 6' x 1/4" 3% OV17 column.

1. L. H. Zalkow, N. Burke, G. Cabat and E. A. Grula, J. Med. and Pharm. Chem., 5, 1342 (1962).
2. L. H. Zalkow, et. al., Tetrahedron, 20, 1419 (1964).
3. L. H. Zalkow and M. Ghosal, J. Org. Chem., 34, 1646 (1969).
4. L. H. Zalkow, et. al., Tetrahedron Letters, 2873 (1972).
5. L. H. Zalkow, N. I. Burke and G. Keen, Tetrahedron Letters, 217 (1964).
6. L. H. Zalkow, et. al., Tetrahedron Letters, 5727 (1968).
7. N. I. Burke, Ph.D. Dissertation, Oklahoma State University, 1966.
8. F. Bohlmann and C. Zdero, Phytochemistry, 15, 1075 (1976).

M.p. 255-257° (uncorrected), rpt.⁹ m.p. 256-257° (266-267° in vacuo); $\nu_{\text{max}}^{\text{KBr}}$ 1710 cm^{-1} , rpt.⁹ $\nu_{\text{max}}^{\text{KBr}}$ 1709 cm^{-1} ; $[\alpha]_{\text{D}} -21^{\circ}$ (C, 1.1 CHCl_3), rpt.⁹ $[\alpha]_{\text{D}} -19$ to -29° ; M^+ m/e 426 (3%). Oxime m.p. 277-278; $[\alpha]_{\text{D}} +58^{\circ}$ (C, 0.91, CHCl_3); rpt.⁹ m.p. 280-282°, $[\alpha]_{\text{D}} +56^{\circ}$. Enol benzoate m.p. 257-258°, $[\alpha]_{\text{D}} +57^{\circ}$ (C, 0.85, CHCl_3), rpt.⁹ m.p. 255-256°, $[\alpha]_{\text{D}} +59^{\circ}$.

Later eluates from the 1:1 hexane-benzene fractions deposited a solid (m.p. 275-277° from hexane, contaminated with friedelin) which was similar to friedelin in the gross features of its i.r. and n.m.r. spectra but showed strong O-H absorption in the i.r. In addition, this solid was indistinguishable from friedelin by glc on a 5% SE-30 column and by t.l.c. on silica gel. However, the solid was clearly distinguishable from friedelin on a 3% OV-17 g.l.c. column and was shown to be friedelan-3 α -ol as follows - Jones oxidation of the alcohol gave friedelin, identical with an authentic sample by g.l.c. (3% OV17 column), i.r., n.m.r. and m.p. The alcohol was converted into its benzoate by heating with benzyol chloride in pyridine. M.p. 248-250° (1:1 CHCl_3 -MeOH), rpt.¹⁰ m.p. 247-248°, the mother liquor yielded a second crop, m.p. 247-248°. Hydrolysis of the benzoate with 8% ethanolic KOH gave friedelan-3 α -ol. M.p. 302-303°, rpt.¹⁰ m.p. 300-301°; $[\alpha]_{\text{D}}^{280} +17$ (C, 1.38 CHCl_3), rpt.¹¹ $[\alpha]_{\text{D}} +18$; $\nu_{\text{max}}^{\text{KBr}}$ 3480, 3610 cm^{-1} ; M^+ m/e 428 (5%), m/e 275 (15%)¹².

Acknowledgements - We express our sincere appreciation to the National Cancer Institute for support of this research (1-R01-CA-18819-01). We also thank Mr. John Novak for preliminary separation and gas chromatographic studies.

9. A. Weizmann, A. Meisels and Y. Mazur, J. Org. Chem., 20, 1173 (1955).
10. V. Anjaneyulu, D. Nagiswara Rao and L. Ramachandra Row, J. Indian Chem. Soc., 44, 123 (1967).
11. W. H. Hui and C. T. Ho, Aust. J. Chem., 1675 (1968).
12. H. Budzikiewics, C. Djerassi and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry - Volume II," Holden-Day, Inc., San Francisco, 1964, pp. 132-136.

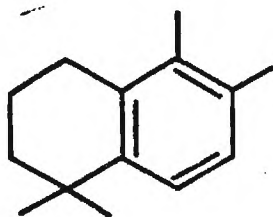
1,2,3,4-TETRAHYDRO-1,1,5,6-TETRAMETHYLNAPHTHALENE.

AN UNUSUAL NATURAL PRODUCT FROM ISOCOMA WRIGHTII.

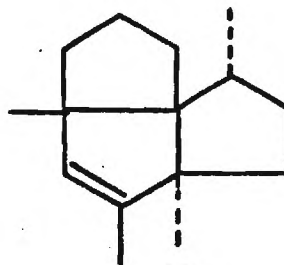
by L. H. Zalkow* and R. N. Harris, III

(School of Chemistry, Georgia Institute of
Technology, Atlanta, Georgia, 30332, U.S.A.)

During the course of continuing studies on the toxic plant rayless goldenrod (Isocoma Wrightii¹), we have isolated the hydrocarbon 1,2,3,4-tetrahydro-1,1,5,6-tetramethylnaphthalene (I) and the evidence for its structure is presented in this communication. The isolation of such an aromatic hydrocarbon from a plant source is unusual and we have been unable to



I



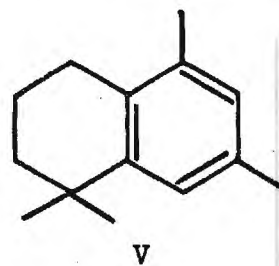
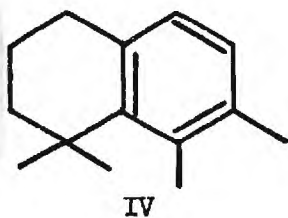
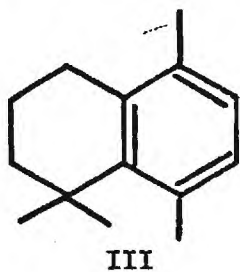
II

uncover any reports in the literature of analogous tetrahydronaphthalenes. Its biogenetic origin raises some interesting questions.

Previous studies on rayless goldenrod have shown it to contain a number of interesting secondary metabolites such as the bacteriostatic agent toxol² and related benzofurans³ and the novel steroids 5 α -androstane 3 β , 16 α , 17 α -triol⁴ and stigmasta-8(14)-22-dien-3 β -ol⁵. Recently, the first reports of the presence of sesquiterpenes in this plant have appeared. Thus, Bohlmann and Zdero⁶ reported the isolation of three 8-oxo- β -cyperons and we have isolated caryophyllene, caryophyllene oxide and a new sesquiterpene of novel skeletal type, isocomer

(II). As discussed later we suggest that 1,2,3,4-tetrahydro-1,1,5,6-tetramethylnaphthalene (I) may arise from a sesquiterpene precursor and hence be of isoprenoid origin rather than acetate or shikimate derived as usual for aromatic natural products.

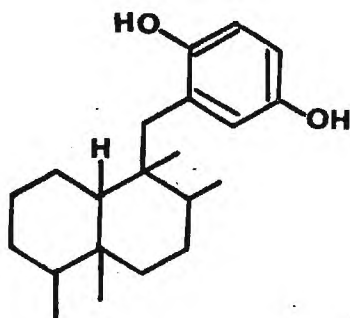
Steam distillation of the hexane extract of the above ground portion of the plant yielded a yellow essential oil (2%), which was distilled at reduced pressure to remove the lower boiling monoterpene fraction. Chromatography of the higher boiling residue on silica gel yielded, in some of the hexane eluents, a homogeneous (g.l.c.) colorless oil, b.p. 75-80°/0.2mm (air bath), isolated in about 2% yield based on steam-volatile oil. On the basis of its mass spectrum (M^+ 188, 18%; m/e 173, 100%, M^+-CH_3) and elemental analysis, the molecular formula $C_{14}H_{20}$ (Calc: C, 89.30; H, 10.70; Found: C, 89.37; H, 10.61) could be assigned to the unknown. That the substance contained a benzene ring was clear from its infrared (ν_{max} 800 cm^{-1}) ultraviolet (λ_{max} = 269,277 nm) spectra and particularly its n.m.r. spectrum which showed that there were only two ortho hydrogens on the benzene ring (δ 6.92; ABq, J = 8 Hz). In addition, the n.m.r. spectrum showed that there were two methyl groups attached to the benzene ring (δ 2.10, 2.24), a gem dimethyl group (δ 1.27, 6 H), two benzylic protons (δ 2.60, t, J = 6 Hz) flanked by two adjacent protons, thus accounting for all of the hydrogen atoms except for two. On the basis of these data structures I, III and IV were considered for this substance.



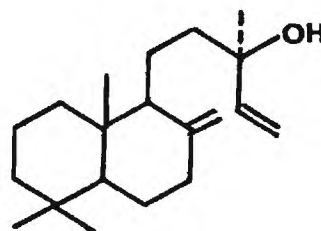
A synthesis of the more symmetrical isomer, III, was therefore undertaken beginning with *p*-xylene by Friedel Crafts acylation with succinic anhydride to give 4(2,5-dimethylphenyl)-4-oxo-butanoic acid, followed by Huang-Minlon reduction to 4(2,5-dimethylphenyl)butanoic acid, then esterification with diazomethane followed by Grignard addition of methylmagnesium iodide to give 2-methyl-5(2,5-dimethylphenyl)pentan-2-ol and finally Friedel-Crafts alkylation with polyphosphoric acid to give 1,1,5,8-tetramethyl-1,2,3,4-tetrahydronaphthalene (III). The *p*-xylene used in the synthesis of III apparently contained a small amount of *m*-xylene which reacted in a parallel series of reactions to give ultimately 1,1,5,7-tetramethyl-1,2,3,4-tetrahydronaphthalene (V). The two tetrahydronaphthalenes III and V were separated by chroma-

tography on silica gel impregnated with silver nitrate (20%), with V being eluted first in hexane-methylene chloride (85:15). Tetralin V was identified by comparison of its i.r. and n.m.r. spectra with those of an authentic synthetic sample prepared in a similar manner⁸. With the i.r. (ν_{\max} 800 cm^{-1}) and mass (M^+ 188, 48%; m/e 173, 100%) spectra of III⁹ were similar to that of the unknown, the two differed by g.l.c. analysis and the differences in the n.m.r. spectra were particularly instructive (III: δ 6.73, s, 2 H; 2.43, s, 3 H; 2.10, s, 3 H; 1.3 s, 6 H). A comparison of the n.m.r. spectrum of V (δ 6.92, bs, 1 H; 6.70, bs, 1 H; 2.23, s, 3 H; 2.13, s, 3 H; 1.25, s, 6H) with that of III and the unknown suggested that the correct structure of the unknown was, in fact, I and not IV because in both the unknown and in V the gem dimethyl group and the two aromatic methyl groups had almost identical chemical shifts, whereas in III both the gem dimethyl group and the aromatic methyl group at C-8 showed rather considerable deshielding as would be expected if the correct structure of the unknown were

A search of the literature revealed that 1,2,3,4-tetrahydro-1,1,5,6-tetramethylnaphthalene (I) had recently been reported as a rearranged degradation product of the sesquiterpene avarol (VI), formed on dehydrogenation with 10% Pd-C at 270°¹⁰. Indeed, the reported spectral properties and a copy of the n.m.r. spectrum verified that our unknown was identical to the above mentioned degradation product¹⁰.



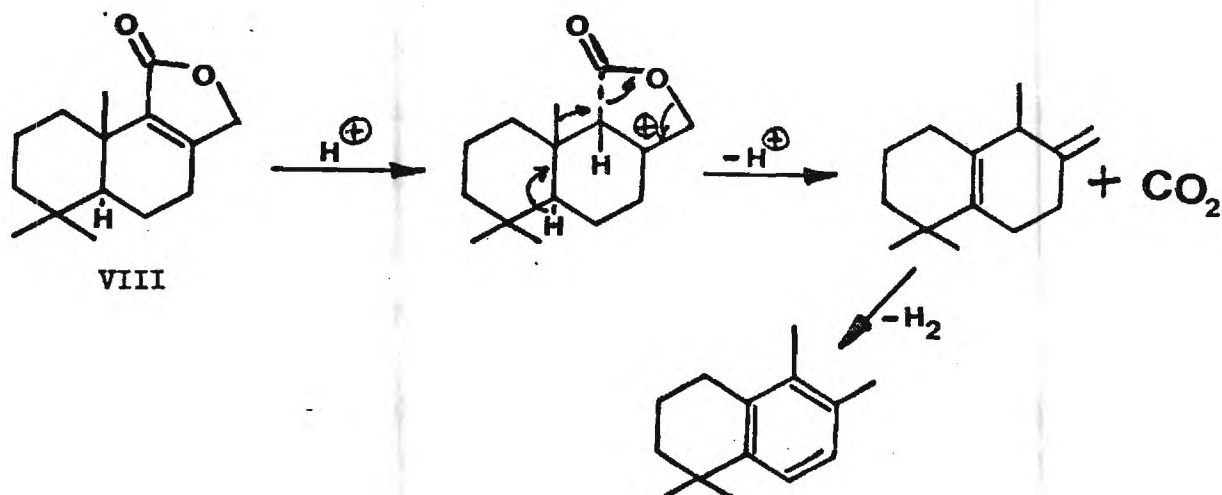
VI



VII

The carbon skeleton of I and its preparation both by the dehydrogenation of avarol, as mentioned above and particularly its reported isolation in the selenium dehydrogenation of manool (VII)¹¹, suggested to us that 1,2,3,4-tetrahydro-1,1,5,6-tetramethylnaphthalene (I

might be biogenetically derived from an isoprenoid precursor and one mechanistically feasible pathway from the known sesquiterpene isodremenin (VIII) is outlined below.



Acknowledgements - We express our sincere appreciation to the National Cancer Institute for support of this research (1-R01-CA-18819-01).

References

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2. L. H. Zalkow, et al., Tetrahedron Letters, 2873 (1972).
3. L. H. Zalkow and M. Ghosal, J. Org. Chem., 34, 1646 (1969).
4. L. H. Zalkow, N. I. Burke and G. Keen, Tetrahedron Letters, 217 (1964).
5. L. H. Zalkow, et al., Tetrahedron Letters, 5727 (1968).
6. F. Bohlmann and C. Zdero, Phytochemistry, 15, 1076 (1976).
7. L. H. Zalkow, R. N. Harris, III, D. Van Derveer and J. A. Bertrand, J.C.S. Chem. Comm., in press.
8. D. Nasipuri, I. De Dalal and D. N. Roy, J. Chem. Soc. Perkin I, 1754 (1973). We are grateful to Professor Nasipuri, Department of Chemistry, Indian Institute of Technology, Kharagur 72 1302, India, for the n.m.r. and i.r. spectra of V.
9. Correct elemental analysis was obtained for this previously unreported isomer.
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THE COOCCURRENCE OF DESMETHYLENCECALIN AND
HYDROXYTREMETONE IN EUPATORIUM RUGOSUM (E. URTICAEOFOLIUM)

L. H. Zalkow*, L. Gelbaum, M. Ghosal and T. J. Fleischmann

School of Chemistry, Georgia Institute of Technology

Atlanta, Georgia 30332, U.S.A.

(Received , Accepted)

Key Word Index - Eupatorium rugosum; compositae; desmethylenecalin (7-hydroxy-2, 2-dimethyl-6-acetyl-2H-chromen); hydroxytremetone (dehydroeuparin; 6-hydroxy-2-isopropenyl-5-acetylcumaranon).

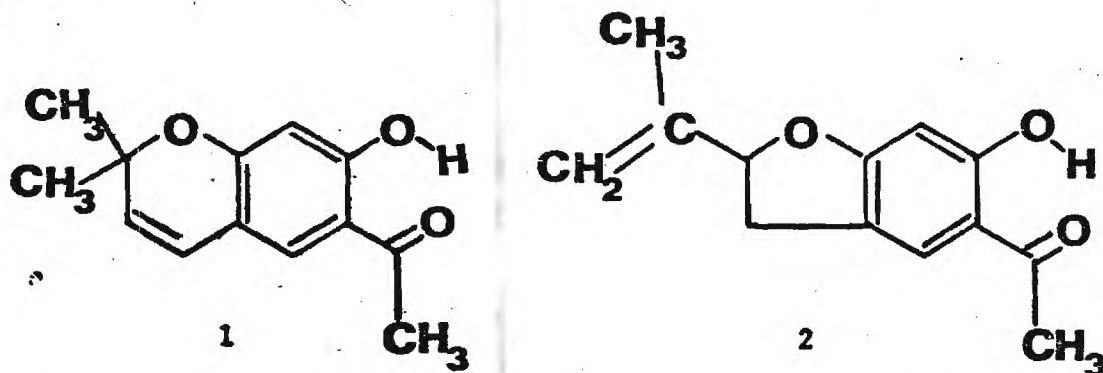
Plant: Eupatorium rugosum collected at Unicoi Gap, Appalachian Trail, Highway 75 north Georgia in September and October, 1976.

Previous Work: Isolation and identification of tremetone, dehydrotremetone and hydroxytremetone¹. Plant responsible for "milk sickness" in cattle and humans^{1,2}.

Present Work: Roots of freshly collected E. rugosum, in bloom, were macerated in a Waring blender with 95% ethanol then continuously extracted with 95% ethanol to give 2% extract based on wet plant. Partition between 5% aqueous hydrochloric acid and chloroform yielded 16% of the ethanol extract in the chloroform layer.

Forty three percent of the chloroform extract was found to be hexane soluble and gas chromatography of the hexane soluble fraction showed five components (OV-17 column), the major component (~50%) being desmethylenecalin (1) and a minor component (~7%) having the same retention time alone and on admixture as authentic hydroxytremetone (2) on both OV-17 and SE-30 columns. Chromatography of the hexane soluble viscous oil on silica gel (230-400 mesh) and elution with hexane gave desmethylenecalin as orange crystals, m.p. 74-76°, rpt.³ m.p. 77°, $\nu_{\text{max}}^{\text{CCl}_4}$ 3400-2500 (broad), 1645 cm^{-1} ; δ (CCl_4) 1.39 (6 H, s), 2.41 (3 H, s), 4.50 (1 H, d, J 10 Hz), 6.21 (1 H, d, J 10 Hz), 6.21 (1 H, s), 7.20 (1 H, s),

1. W. A. Bonner and J. I. DeGraw, Jr., *Tetrahedron*, **18**, 1295 (1962).
2. J. F. Couch, *J. Agr. Res.*, **35**, 547 (1927); *J. Am. Med. Assoc.* **91**, 234 (1928); *J. Am. Chem. Soc.*, **51**, 3617 (1929).
3. F. Bohlmann and M. Grenz, *Chem. Ber.* **103**, 90 (1970).



12.44 (1 H, s); MS: M^+ 218 (16%), 204 (100%), 202 (12%), 185 (10%). Identical with i.r. and ^1H n.m.r. spectra of an authentic sample of desmethylenececalin⁴.

Hydroxytremetone (2) was more readily isolated as follows. The ethanol extract (138 g) of the above ground portion of the green plant was steam-distilled and the aqueous solution and insoluble portion were successively extracted with hexane, ether and finally chloroform, then the water solution was filtered and evaporated to yield 91 g of residue. This residue was refluxed in 2N aqueous-ethanolic hydrochloric acid, then extracted with ether to give 15 g of residue which was refluxed in 10% ethanolic sodium hydroxide. Extraction of the latter solution with ether, washing with water, drying and evaporation gave 0.7 g of residue which was chromatographed on silica gel (100-200 mesh) to give 190 mg of almost pure hydroxytremetone in the hexane-ethyl acetate (4:1) eluent. Analytically pure (single peak by gas chromatography on SE-30 column) hydroxytremetone (2) was obtained by rechromatography on silica gel (100-200 mesh) and crystallization from hexane. M.p. 63-65°, rpt. m.p. 70-71°¹, 69°²; $[\alpha]_D^{25}$ - 43° (C, 0.40, EtOH), rpt. $[\alpha]_D^{24}$ - 50.7° (C, 0.74, EtOH); $\nu_{\text{max}}^{\text{CCl}_4}$ 1642; λ_{max} (EtOH) 208, 213, 232, 237, 280 and 335 nm; δ (CDCl_3) 1.75 (3 H, s), 2.53 (3 H, s), 2.95 (1 H, ddd, J 15, 9, 1), 3.29 (1 H, ddd, J 15, 9, 1), 6.32 (1 H, s), 7.48 (1 H, s), 12.98 (1 H, s); M.S.: Calc. for $\text{C}_{13}\text{H}_{14}\text{O}_3$: 218.0943, found 218.0965; M^+ 218 (100), 203 ($M^+ - \text{CH}_3$, 72%), 175 ($M^+ - \text{CH}_3\text{CO}$, 42%). The u.v.¹, i.r.³, ^1H -n.m.r.³ and M.S.³ were identical, within experimental error, with the values reported in the literature and the i.r. and n.m.r. spectra were identical, within experimental errors, to spectra of an authentic sample⁴.

To our knowledge, the only prior report of the occurrence of desmethylenececalin has been in *Helianthella uniflora* where it cooccurs with hydroxytremetone³.

⁴ We thank Prof. Dr. F. Bohlmann, Institut für Organische Chemie der Technischen Universität Berlin, for these spectra.

Whereas hydroxytremetone has previously been reported in E. rugosum¹, its cooccurrence with desmethylenecalin is of particular phytochemical interest.

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ISOCOMENE¹. A NOVEL SESQUITERPENE FROM ISOCOMA WRIGHTII[‡].

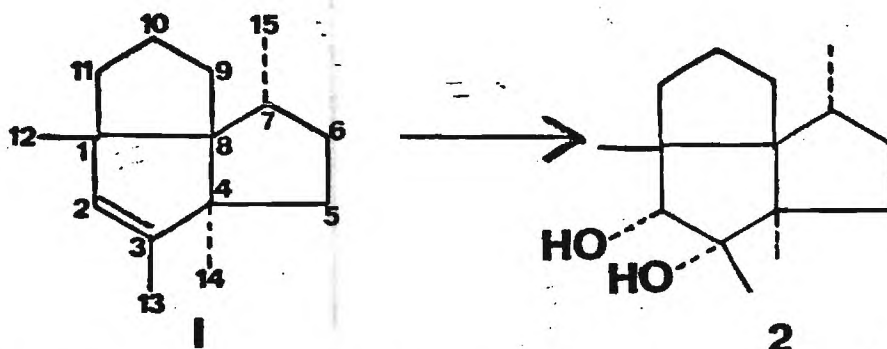
X-RAY CRYSTAL STRUCTURE OF THE CORRESPONDING DIOL.

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Summary A new sesquiterpene (isocomene, 1) representing a novel skeletal type has been isolated from the toxic² plant *Isocoma Wrightii*[‡]. Its structure was confirmed by X-ray analysis of its corresponding diol (2).

Rayless goldenrod (*Isocoma Wrightii*[‡]) has a notorious past as a plant toxic to cattle and sheep² but the exact nature of the toxin remains unresolved. The



plant has been shown to contain the bacteriostatic agent toxol^{2,3} and related benzofurans⁴, the novel steroids 5 α -androstan-3 β , 16 α , 17 α -triol⁵ and stigmasta-8(14), 22-dien-3 β -ol⁶. Only recently have sesquiterpenes been found in *I. Wrightii*. Thus, Bohlmann and Zdero⁷ reported the presence of three 8-oxo- β -cyperons in the roots and we have isolated caryophyllene and caryophyllene oxide from the stems and leaves⁸. We wish to report here the isolation and structure of a new sesquiterpene of novel skeletal type from *I. Wrightii* and have given it the trivial name isocomene (1).

[‡] Formerly known as *Haplopappus heterophyllus*. D. S. Correll and M. C. Johnston, "Manual of Vascular Plants of Texas," Texas Research Foundation, Renner, Texas, 1970.

Isocomene (1) was isolated from the dried stems and leaves by extraction with hexane or from the saponified methanol extract by steam distillation followed by fractional distillation. The fraction of b.p. 65-75°/0.05 mm was shown by gas chromatography to be composed of approximately 90% caryophyllene and 10% isocomene (1). Chromatography on silica gel impregnated with silver nitrate (20%) gave isocomene (1) in the hexane-methylene chloride (95:5) eluent as a colorless oil, b.p. 65-70°/0.35 mm (bath). Calcd. for $C_{15}H_{24}$: C, 88.32; H, 11.86 Found: C 88.11; H, 11.88. Mass spectrometry: m/e 204 (M^+ , 15%), 189 (19%), 162 (100%), 147 (42%), 119 (35%); $\nu_{\max}^{CCl_4}$ 3020, 1670, 840 cm^{-1} ; δ 0.87 (3 H, d, J 7 Hz), 1.02 (6 H, s), 1.67 (3 H, d, J 1.5 Hz), 4.83 (1 H, m); ^{13}C n.m.r.: δ 56.4, 59.7, 63.6 (s, quaternary C's), 132.1 (d, olefinic C with single H), 142.1 (s, olefinic C with no H).

Mass spectrometry, elemental analysis and the 1H and ^{13}C n.m.r. spectra indicated that isocomene (1) was tricyclic, contained a trisubstituted double bond with an attached methyl group, two additional methyl groups attached at quaternary carbons, a methyl group at a tertiary carbon and finally a quaternary carbon bearing no methyl groups. By analogy to known sesquiterpene skeletons⁹ the six proton n.m.r. singlet at δ 1.02 was assumed to arise from a gem dimethyl group and only after the X-ray analysis, described below, was it clear that, in fact, this was not the case and isocomene actually represented a previously unknown sesquiterpene skeleton.

On treatment with osmium tetroxide in pyridine, isocomene gave a mixture of diols from which the isomer (2), was separated by crystallization from pentane-ether and chromatography on silica gel, M.P. 134-136°; Calcd. for $C_{15}H_{26}O_2$: C, 75.63; H, 10.92. Found: C, 75.54; H, 11.04. Mass spectrometry: m/e 238 (M^+ , 2%), 220 ($M^+ - H_2O$, 29%), 134 (30%), 122 (90%), 109 (100%); $\nu_{\max}^{CDCl_3}$ 3540, 3590 cm^{-1} ; δ 0.91 (3 H, d, J 6.5 Hz), 0.94 (3 H, s), 1.03 (3 H, s), 1.15 (3 H, s), 3.50 (1 H, d, J 8 Hz). The structure of diol (2) and "ipso facto," the structure of isocomene (1) was firmly established by a single crystal X-ray analysis. The diol crystallized from pentane in the orthorhombic space group $P 2_1 2_1 2_1$ with $a = 6.898$ (2), $b = 12.397$ (6), $c = 16.042$ (6) Å, $Z = 4$. The intensity data were measured with a Syntex P2, four-circle diffractometer, equipped with a graphite monochromator, using θ -2 θ scan technique. The structure, which was solved by direct methods, was refined by least-squares methods to convergence of $R = 0.069$ for 1046 reflections with $I > 3\sigma(I)$. Variables included a scale factor, coordinates of all carbon and oxygen atoms, anisotropic thermal parameters for oxygen and selected

carbon atoms and isotropic thermal parameters for the remaining carbon atoms. However, parameters were not varied for hydrogen atoms; fixed thermal parameters of 5.0 were used. The structure of the diol is illustrated in Figure 1.

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