Tetrahedron Letters No.7, pp. 497-500, 1970. Pergamon Press. Printed in Great Britain.

ISOLATION OF THE ELUSIVE (-)-GERMACRENE-A FROM THE GORGONIAN, *EUNICEA MAMMOSA*

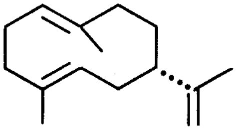
CHEMISTRY OF COELENTERATES. XVIII.[1]

Alfred J. Weinheimer, William W. Youngblood,
Paul H. Washecheck, Tom K. B. Karns and Leon S. Ciereszko

Department of Chemistry, The University of Oklahoma, Norman, Oklahoma 73069

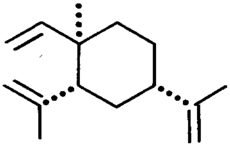
(Received in USA 15 November 1969; received in UK for publication 9 January 1970)

We wish to report the isolation of the long sought sesquiterpene, germacrene-A (I),[2] from the gorgonian *Eunicea mammosa* Lamouroux taken at Bimini, The Bahamas. Our earlier attempt[3] to isolate this compound from the same source was frustrated by its exceptional thermal lability toward the Cope rearrangement to β-elemene (II). At slightly elevated temperatures (I) isomerizes at an appreciable rate, and is unstable even in freezer storage over extended periods. Attempted catalytic hydrogenation also resulted in extensive prior isomerization to β-elemene. Such rearrangements of cyclodeca-1,5-dienes are now well documented,[4,5,6] and the working hypothesis that elemene derivatives generally are artifacts formed by thermal isomerization of natural germacrene percursors during isolations appears to be widely accepted.[6,7,8]



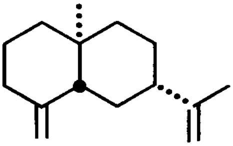
I

(-)-Germacrene-A



II

(+)-β-Elemene



III

(-)-β-Selinene

Freshly collected specimens of *E.mammosa* were air dried for one day at Bimini, and after four days in transit to this laboratory, were immediately stored in the deep freeze. The hydrocarbon fraction (0.8% dry weight) was isolated by two leachings with hexane (25°), followed by rapid concentration at temperatures less than 35° and subsequent chromatography of the residue in hexane on Florisil. The hydrocarbons could be stored without change in carbon tetrachloride solution at Dry Ice temperatures. Gas chromatography (5% Carbowax 20 M, column at 110°, injector heater off) revealed the presence of four components. The first (unidentified) and third (β-selinene, III) constituted less than 2% of the total. The second and fourth components were subsequently identified as (+)-β-elemene (40%) and (-)-germacrene-A (60%), respectively.

Chromatographic separation of germacrene-A from this mixture was complicated by its instability toward silicic acid adsorbents. Though stable to Florisil, this adsorbent typically displayed no selectivity for the individual hydrocarbons. Florisil impregnated with silver nitrate also was unsatisfactory since it continuously isomerized the slower moving β-elemene to the faster moving β-selinene which was incompletely resolved from germacrene-A (which, however, appeared to be stable on this adsorbent). This dilemma was successfully resolved by use of an adsorbent mixture consisting of 30% powdered sucrose (confectioners sugar, 3% cornstarch) and 70% Florisil mixed as dry solids in a blendor in hexane. (An adsorbent of similar composition prepared by evaporation of an aqueous sucrose solution on Florisil in the usual way showed neither selectivity nor adsorptivity.)

Chromatography of the hydrocarbon mixture (4 g) on this adsorbent (540 g) in a water cooled column (5°) with 2% benzene in hexane (each dried over calcium hydride) under 2.5 psi nitrogen pressure afforded pure germacrene-A (385 mg) in fractions 50 to 65 (25 ml per fraction, 5 ml per min): n_D^{25} 1.5089; $[\alpha]_D^{25}$ -3.2° (c=14.4, CCl_4), -2.8° (c=6.15, CCl_4), mw 204 (ms). Its infrared spectrum (thick and thin films, Fig. 1) showed significant bands at 3060, 1660 and 880 (terminal $=CH_2$), 1660 and 840 cm^{-1} (trisubstituted double bonds). Its nmr spectrum (CCl_4, Fig. 2) displayed a broad absorption at δ 4.75-5.40 (2H, vinyl protons on trisubstituted double bonds), a distorted "doublet" at δ 4.60 identical in shape with that shown by β-elemene (2H, terminal $-CH_2$) and vinyl methyl absorptions at δ 1.70 and 1.48. It showed no selective ultraviolet absorption above 200 nm. Gas chromatography under the conditions described above showed a single peak[9] corresponding to the fourth component of the original mixture. With the injector temperature at 240°, a single peak was again observed, now corresponding to the second component, β-elemene. After heating three hours at 120° under nitrogen, germacrene-A was isomerized completely to β-elemene (vpc and nmr).

The hydrocarbon fraction isolated by distillation of the residue from the hexane extract at

120° (0.1 mm) consisted almost entirely (99%) of (+)-β-elemene which was purified by preparative gas chromatography (SE-30): n_D^{25} 1.4910, d_4^{20} 0.8869, $[\alpha]_D^{25}$ 15.1° (neat), mw 204 (ms). Its identity was established by the excellent correspondence of its physical properties[10] and infrared spectrum[11] with published information, and the compatability of its nmr spectrum with the assigned structure. The absolute configuration shown in (II) for (+)-β-elemene follows from the synthesis of (-)-β-elemene from (-)-elemol[12], which has been correlated with saussurea lactone of known absolute configuration.[13] The quasi-chair transition state for the Cope rearrangement[14] leads to the absolute configuration shown in (I) for (-)-germacrene-A.

It is noteworthy that (+)-β-elemene is enantiomeric with the common terrestrial form of this hydrocarbon, a relationship observed previously for the sesquiterpene hydrocarbons derived from another gorgonian.[15] Irie et al.[16] have isolated the (+)-enantiomer from another marine source, a brown alga. The only other reported source of this enantiomer is the essential oil of the fruit of a Transcaucasian mountain parsley.[17] It can be anticipated that most terrestrial sources of germacrene-A will elaborate the (+)-enantiomer.

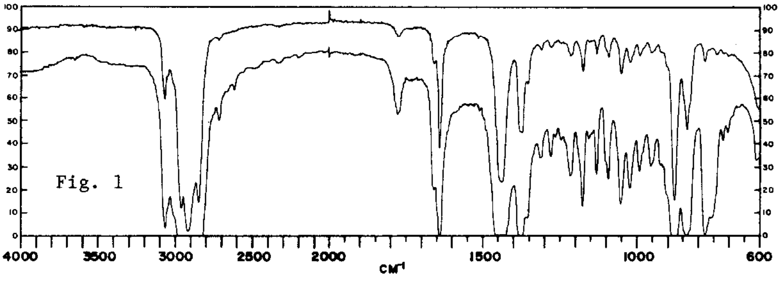
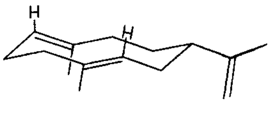


Fig. 1



(-)-germacrene-A

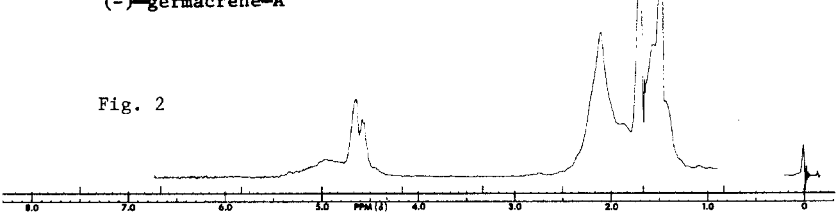


Fig. 2

Acknowledgements: We are grateful to Robert F. Mathewson, Director, of the Lerner Marine Laboratory operated by the American Museum of Natural History, for use of collecting facilities at Bimini, to the National Institutes of Health for generous financial support (grants GM-08003 and HE-05675) and to the National Science Foundation for partial purchase of an nmr instrument (grant FM-830).

References and Footnotes

1. The two preceding papers in this series, XVI by A. J. Weinheimer and R. L. Spraggins, and XVII by F. J. Schmitz, E. D. Lorance and L. S. Ciereszko, were presented at the *"Food-Drugs from the Sea"* Conference at the University of Rhode Island, Kingston, August 24-27, 1969, and are to appear in the Transactions of that meeting, published by the Marine Technology Society, in press.

2. For nomenclature of the germacrenes, see K. Morikowa and Y. Hirose, *Tetrahedron Letters*, 1799 (1969).

3. A. J. Weinheimer, F. J. Schmitz and L. S. Ciereszko, "Drugs From the Sea," *Transactions of the Drugs from the Sea Symposium*, Marine Technology Society, 135 (1968).

4. G. H. Kulkarni, G. R. Kelkar and S. C. Bhattacharyya, *Tetrahedron*, **20**, 1301 (1964).

5. N. H. Fischer, T. J. Mabry and H. B. Kagan, *Tetrahedron*, **24**, 4091 (1968).

6. R. V. H. Jones and M. D. Sutherland, *Aust. J. Chem.*, **21**, 2255 (1968).

7. R. V. H. Jones and M. D. Sutherland, *Chem. Commun.*, 1229 (1968).

8. K. Nishimura, N. Shimoda and Y. Hirose, *Tetrahedron Letters*, 3097 (1969).

9. The base line of the chromatogram showed a sharp positive step to a new level at precisely the retention time of β-elemene, and returned to the original level after elution of the germacrene-A peak. As expected for a steady state formation of β-elemene, the height of this step increased at higher oven temperatures.

10. O. Motl and V. Lukes, *Coll. Czech. Chem. Commun.*, **27**, 987 (1962).

11. F. Sorm, M. Holub, R. Sykora, J. Mleziva, M. Streibl, J. Pliva, B. Schneider, and V. Herout, *Coll. Czech. Chem. Commun.*, **18**, 512 (1953).

12. V. Sykora, V. Herout, and F. Sorm, *Coll. Czech. Chem. Commun.*, **21**, 267 (1956).

13. A. D. Wagh, S. K. Paknikar, and B. C. Bhattacharyya, *Tetrahedron*, **20**, 2647 (1964).

14. W. von E. Doering and W. R. Roth, *Tetrahedron*, **18**, 67 (1962).

15. A. J. Weinheimer, P. H. Washecheck, D. van der Helm and M. B. Hossain, *Chem. Commun.*, 1070 (1968).

16. T. Irie, K. Yamamoto, and T. Masamune, *Bull. Chem. Soc. Japan*, **37**, 1053 (1964).

17. G. V. Pigulevskii and A. V. Borovkov, *J. Gen. Chem. USSR*, **32**, 3054 (1962).