hexane) (see also Fig. 1 of ref. 1): $[\alpha]_{589}$ + 268°; $[\alpha]_{284}$ + 13,000°; $[\alpha]_{258}$ ± 0°; $[\alpha]_{234}$ −11,000°; $[\alpha]_{228}$ −6800°.

Tetracyanoethylene Adduct of I.—Approximately 80 mg. of I was treated at room temperature with a solution of 79.2 mg. of tetracyanoethylene in 1.0 ml. of freshly distilled tetrahydrofuran. The mixture turned red immediately; on standing for several days it became yellow. It was concentrated under reduced pressure, and the residue was taken up in 8 ml. of hot hexane. On cooling, the adduct separated in long needles, which were recrystallized from hexane-benzene; m.p. 109–111°.

Anal. Calcd. for $C_{17}H_{14}N_4$: C, 74.45; H, 5.11; N, 20.44. Found: C, 74.56; H, 5.11; N, 20.55.

Acknowledgment.—The authors are much indebted to Professor K. Biemann, M.I.T., for permission to quote the results of his mass-spectrographic investigation of I, and to Dr. E. D. Becker and Mr. R. B. Bradley of this Institute for the NMR data.

Steroids and Related Natural Products. XII. *Fomes applanatus*[1,2]

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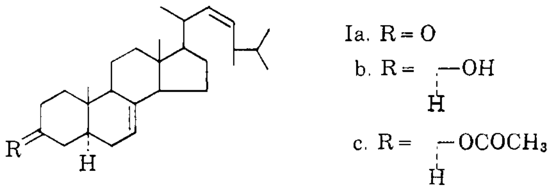
The Polyporaceae family (Agaricales order) contains tube-bearing basidiomycetous fungi of a fleshy-tough to woody consistency. Members of this family are wood-inhabiting annuals or perennials commonly known as bracket or shelf fungi.[3] Interest in locating new sources of lanostane-type tetracyclic triterpenes has led us to begin a phytochemical survey of the Polyporaceae genus *Fomes*.[4,5]

Although *Fomes* species have been recognized since 1878,[3] preliminary chemical examinations of only six, apparently, have been described.[6] One of these, *Fomes applanatus*, was reported to contain a pigment, mannitol and "ergosterol-like compounds."[6a] Our initial study has been concerned with evaluating *Fomes applanatus* as a source of lanostane derivatives.

Specimens of *Fomes applanatus*[7,8] collected in northeastern Maine, were successively extracted with petroleum ether, diethyl ether, and 95% ethanol. A small quantity of crystalline material which separated from the petroleum ether extract yielded 3-oxo-24S-methyl-5α-cholesta-7,22-diene (Ia)[9] and 3β-hydroxy-24S-methyl-5α-cholesta-7,22-diene (Ib)[10] following chromatographic separation on activated alumina. The remaining hydrocarbon extract was saponified and small amounts of sterols Ia and Ib were again isolated from the neutral components. Tentative identification of these substances was based on elemental analyses, physical constants, and their infrared spectra. The structures were confirmed by comparison with authentic samples prepared from ergosterol.



Both the disaccharide trehalose[11] and 5α-dihydroergosterol (Ib) were eventually isolated from the 95% ethanol extracts. However, attempts at isolating in a pure form reasonable quantities of other neutral (or acidic) sterols from the petroleum ether, diethyl ether, or ethanol extracts were unsuccessful. For example, separation of the petroleum ether extract based on isolating reactive carbonyl compounds with Girard's reagent T and sterols with digitonin held no particular advantage.

Similar evaluation of other *Fomes* species is now in progress.

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[1] Part XI, G. R. Pettit and D. M. Piatak, *J. Org. Chem.*, **27**, 2127 (1962).

[2] This investigation was supported by PHS Research Grants CY-4074(C2) and CY-4074(C3) from the National Cancer Institute, Public Health Service and in part by National Science Foundation Research Grant G-19500.

[3] North American Polyporaceae, for example, comprise 8 genera and approximately 235 species: L. O. Overholts, "The Polyporaceae of the United States, Alaska and Canada," University of Michigan Press, Ann Arbor, Michigan, 1953.

[4] Isolation of the polyporenic acids from several *Polyporus* species (annuals) suggested that the closely related *Fomes* genera might also produce unusual lanostane derivatives. A summary of the elegant structural studies involving polyporenic acids has been prepared by E. R. H. Jones and T. G. Halsall, "Progress in the Chemistry of Organic Natural Products," Vol. XII, L. Zechmeister, ed., Springer-Verlag, Vienna, 1955, p. 44; *cf.* also, J. J. Beereboom, H. Fazakerley, and T. G. Halsall, *J. Chem. Soc.*, 3437 (1957).

[5] A distinguishing characteristic of this genus is the perennial nature of its species. Certain *Fomes* have been known to survive seventy years.

[6] Refer to: (a, *F. applanatus*), J. Zellner, *Monatsh. Chem.*, **36**, 611 (1915) [*Chem. Abstr.*, **9**, 3083 (1915)]; (b, *F. fomentarius*), H. R. Arthur, T. G. Halsall and R. D. Smith, *J. Chem. Soc.*, 2603 (1958); (c, *F. hartigii*), L. Canonica and A. Fiecchi, *Gazz. chim. ital.*, **89**, 818 (1959) [*Chem. Abstr.*, **54**, 22711 (1960)]; (d, *F. juniperinus*), M. Anchel, A. Hervey, and W. J. Robbins, *Proc. Natl. Acad. Sci.* (U.S.), **38**, 655 (1952) [*Chem. Abstr.*, **47**, 4957 (1953)]; (e, *F. officinalis*), R. M. Gascoigne, J. S. E. Holker, B. J. Ralph, and A. Robertson, *J. Chem. Soc.*, 2346 (1951) and J. Valentin and S. Knutter, *Pharm. Zentralhalle*, **96**, 478 (1957) [*Chem. Abstr.*, **52**, 13187 (1958)]; and (f, *F. pinicola*), T. Shibamoto, K. Minami, and T. Tajima, *J. Japan Forest Soc.*, **35**, 56 (1953) [*Chem. Abstr.*, **48**, 6993 (1954)].

[7] This cosmopolitan species variously known as *Boletus* or *Polyporus applanatus* and as *Ganoderma applanatum*, *Polyporus megaloma*, or *Elfvingia megaloma* is most frequently encountered in hardwood regions. Samples measuring from *ca.* ten to forty cm., at the widest point along their horizontal plane, were used in this study.

[8] We are indebted to Dr. Martin A. Rosinski, Department of Botany and Plant Pathology, University of Maine, for identifying the plant material and for valuable counsel with several botanical problems.

[9] The 24-methyl of 5α-dihydroergosterol (Ib) has been assigned a β_F configuration by K. Tsuda, Y. Kishida, and R. Hayatsu, *J. Am. Chem. Soc.*, **82**, 3396 (1960). Employing the valuable system proposed by R. S. Cahn, C. K. Ingold, and V. Prelog, *Experientia*, **12**, 81 (1956), this position may now be designated S. Ketone Ia has also been isolated from a specimen of *Fomes fomentarius* (see footnote 6b).

[10] Recently, sterol Ib was found to be a constituent of *Polyporus pinicola:* T. G. Halsall and G. C. Sayer, *J. Chem. Soc.*, 2031 (1959).

[11] Isolation of trehalose from *Fomes pinicola* has been reported by T. Shibamoto, K. Minami, and T. Tajima, *J. Japan Forest Soc.*, **34**, 390 (1952).

Experimental[12,13]

General Extraction Procedure.—In a typical experiment, specimens of *Fomes applanatus*, collected (by Drs. B. Green, T. R. Kasturi, and the senior author, June, 1960) approximately 2–3 miles west of Duck Pond, Hancock County, Maine, were air-dried at room temperature, stripped of adhering bark, and ground to a coarse powder. A 2.85-kg. sample of the fungus sporophore was exhaustively extracted with hot petroleum ether (b.p. 65–70°). The total extract of yellow oil weighed 26 g. A substantial portion (17.5 g.) of this material could be obtained using a room-temperature procedure. Following treatment with petroleum ether the fungus was dried, and the extraction procedure was repeated successively with refluxing diethyl ether and 95% ethanol. The ether extract led to a viscous brown oil weighing 5.0 g. and the ethanol extract gave 92 g. of dark gum.

Other specimens of *Fomes applanatus* collected by the authors south of Nicatous Lake (November, 1960) and near the Pistol lakes (September, 1961) of Maine (Hancock County) gave lesser yields of the respective solvent extracts. Material from these sources was employed whenever larger quantities of compounds described in the sequel were needed.

Isolation of 3β-Hydroxy-24S-methyl-5α-cholesta-7,22-diene (Ib) and 3-Oxo-24S-methyl-5α-cholesta-7,22-diene (Ia).—The crystalline material which slowly (several days) separated from the crude petroleum ether extract (26 g.), described above, was collected and recrystallized from petroleum ether; yield 0.4 g. of colorless needles, m.p. 123–148°. An 0.80-g. specimen corresponding to this fraction was chromatographed in petroleum ether on activated alumina.[14] Elution with 1:2 petroleum ether–benzene gave **3-oxo-24S-methyl-5α-cholesta-7,22-diene** (0.12 g.) as platelets melting at 181–183°. The fraction (Ib, 0.40 g.) eluted with 4:1 benzene–chloroform recrystallized from ethanol as plates, m.p. 170–171°. The latter specimen proved to be **5α-dihydroergosterol (Ib)**. Both ergosterol derivatives were identical[15] with authentic samples.[6b,16]

Following removal of crystalline material from the original extract it was heated 3 hr. in a refluxing solution composed of ethanol (125 ml.) and 5 *N* aqueous sodium hydroxide (125 ml.). The solution was cooled and extracted with ether. The aqueous phase was acidified and acidic products were methylated with diazomethane. A cursory examination of the methyl esters, using gas chromatographic methods, indicated that this mixture was composed of at least eleven fatty acids.

Removal of solvent from the neutral ethereal solution (*see above*) yielded an orange-red residue (4.59 g.). A portion of this mixture crystallized from methanol as pale yellow plates (0.44 g.), m.p. 162–165°. A larger quantity (1.0 g.) of the crystalline material was prepared and acetylated (1:1 acetic anhydride–pyridine, 2 hr. at reflux). The solid product which separated upon cooling the reaction mixture was collected, washed with ethanol, and recrystallized from the same solvent to yield plates (0.7 g.) melting at 173–177°. Four recrystallizations from ethanol raised the melting point to 181–182°. The sterol (Ib, 0.7 g.) was further purified by treatment (8 hr.) with maleic anhydride (0.35 g.) in refluxing zylene (20 ml.). Following removal of solvent, *in vacuo*, the yellow residue was heated (2 hr.) in refluxing methanol (60 ml.) containing potassium hydroxide (2.5 g.). The mixture was diluted with ether and washed with aqueous sodium bicarbonate and water. After removing the dry (magnesium sulfate) solvent, residual material was recrystallized from chloroform–methanol; yield 0.6 g. Four recrystallizations from chloroform–methanol gave a pure specimen of **5α-dihydroergosterol** melting at 174–176° (lit.,[17] m.p. 176°). An analytical sample was dried 24 hr. at 130° and 48 hr. at 100°, *in vacuo*.

Anal. Calcd. for $C_{28}H_{46}O$: C, 84.35; H, 11.63; active H, 0.25. Found: C, 83.81; H, 11.53; active H, 0.36.

The acetate derivative (Ic, prepared using 1:1 acetic anhydride–pyridine, 2-hr. reflux period) recrystallized from chloroform–methanol as plates melting at 181–182° (lit.,[18] m.p. 180–182° and 184–186.8°).

Anal. Calcd. for $C_{30}H_{48}O_2$: C, 81.76; H, 10.98. Found: C, 81.86; H, 10.66.

The sterol Ib and its acetate derivative were identical[15] with authentic specimens prepared[6b,16] from ergosterol.

The methanol mother liquor remaining after crystallization of sterol Ib was concentrated to a viscous orange oil (4.15 g.). A petroleum ether solution of this product (9.9 g., from several pooled experiments) was chromatographed on activated alumina.[19] A fraction (0.27 g.) eluted by 2:1 petroleum ether–benzene partially crystallized (0.18 g., m.p. 173–176°) on standing. Repeated recrystallization from chloroform–methanol yielded a pure sample of **3-oxo-24S-methyl-5α-cholesta-7,22-diene**, m.p. 181–183° (lit.,[6b] m.p. 183–185°), $[\alpha]^{21}\text{D}$ 0.0° (*c*, 0.81). RD in dioxane (*c*, 1.69), 22–24°; $[\alpha]_{296}$ −70°, $[\alpha]_{317}$ +59° and $[\alpha]_{370}$ +1.8°.

Anal. Calcd. for $C_{28}H_{44}O$: C, 84.78; H, 11.18. Found: C, 84.57; H, 11.03.

Ketone Ia was identical[15] with an authentic sample (m.p. 185–186°) prepared[6b] from 5α-dihydroergosterol. A pure specimen of the **2,4-dinitrophenylhydrazone** derivative crystallized from chloroform–ethanol as yellow needles, m.p. 212–215°, $[\alpha]^{22}\text{D}$ 0.0° (*c*, 0.49).

Anal. Calcd. for $C_{34}H_{48}O_4N_4$: C, 70.80; H, 8.39; N, 9.71. Found: C, 70.79; H, 8.40; N, 9.80.

A combination of column and thin layer chromatographic methods indicated that further separation of the residual neutral product would most probably be unprofitable.

Isolation of Trehalose.—When the 95% ethanol extract (*vide supra*) of *Fomes applanatus* was concentrated to *ca.* 350 ml., a yellow-brown solid (4.5 g.) separated (on standing). After collecting this material, the filtrate slowly deposited large colorless prisms (5.2 g.) of *trehalose hydrate*, m.p. 94–95°. Four recrystallizations from water–ethanol gave a pure sample melting at 95–97° (lit.,[20] m.p. 97–98°). Thin layer chromatography of this substance on Kieselgel G using 4:4:2 isopropyl alcohol–ethyl acetate–water[21] supported its homogeneity. Mixture melting point determination and both thin layer chromatographic and infrared spectral comparison with a commercial (Nutritional Biochemicals Corp.) sample of trehalose hydrate (m.p. 95–97°) confirmed the structural assignment.

Drying at 100° for 24 hr., *in vacuo*, gave **anhydrous trehalose**, $[\alpha]^{21}\text{D}$ +194° (water; *c*, 1.14).[22]

Anal. Calcd. for $C_{12}H_{22}O_{11}$: C, 42.10; H, 6.48; O, 51.42. Found: C, 42.04; H, 6.48; O, 50.92.

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[12] Melting points are uncorrected and were observed employing a Fisher-Johns melting point apparatus. Infrared and RD measurements were recorded by Dr. R. A. Hill of this laboratory. The latter were accomplished using 1.0-dm. annealed quartz cells and the optical rotatory dispersion accessory ($\theta = 79°44'$) for the Perkin-Elmer Model 4000A spectrophotometer. Optical rotation (chloroform solution) measurements at the sodium D line were provided by Drs. Weiler and Strauss, Oxford, England. Elemental analyses were performed in the laboratory of Dr. A. Bernhardt, Mülheim, Germany.

[13] We wish to acknowledge the contributions of Dr. T. R. Kasturi during a preliminary investigation of *Fomes applanatus* solvent extracts.

[14] Merck aluminum oxide "suitable for chromatography."

[15] Established by mixture melting point determination and infrared comparison in potassium bromide.

[16] G. D. Laubach and K. J. Brunings, *J. Am. Chem. Soc.*, **74**, 705 (1952).

[17] D. H. R. Barton and J. D. Cox, *J. Chem. Soc.*, 1354 (1948). *Cf.* also, ref. 6b and 16.

[18] R. C. Anderson, R. Stevenson, and F. S. Spring, *J. Chem. Soc.*, 2901 (1952) and W. V. Ruyle, E. M. Chamberlin, J. M. Chemerda, G. E. Sita, L. M. Aliminosa, and R. L. Erickson, *J. Am. Chem. Soc.*, **74**, 5929 (1952). See also footnote 6b.

[19] Aluminum Co. of America's grade F-20.

[20] R. V. Lemieux and H. F. Bauer, *Can. J. Chem.*, **32**, 340 (1954).

[21] E. Stahl and U. Kaltenbach, *J. Chromatog.*, **5**, 351 (1961).

[22] C. S. Hudson, *J. Am. Chem. Soc.*, **38**, 1566 (1916), reported $[\alpha]\text{D}$ +197°.

Subjecting a 42-g. sample of the remaining 95% ethanol extract to basic hydrolysis (250 ml. of 5% aqueous sodium hydroxide) over a 4.5-hr. period, in refluxing ethanol (250 ml.), led to 1.9 g. of neutral material and a large quantity of dark polymer. Following acid hydrolysis of another 42-g. portion of ethanol extract, using 240 ml. of 6% hydrochloric acid and the same general procedure noted directly above, a 4.3-g. neutral fraction was isolated. Thin layer chromatograms of both neutral products on silicagel G in 9:1 benzene–ethyl acetate indicated that each was a complex mixture. Partial resolution of the neutral material from acid hydrolysis using column chromatography on activated alumina[14] substantiated this observation. However, a crude specimen (0.24 g., m.p. 162–168°) of 5α-dihydroergosterol was eventually isolated from the 1.9-g. sample of neutral product by fractional recrystallization (methanol–chloroform as solvent).

The Reaction of Vinyl Sulfides with Alkyllithium. II[1]

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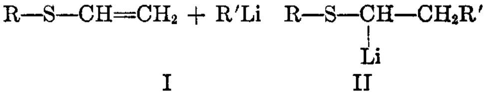
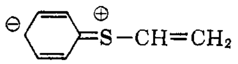
Received March 1, 1962

We have extended our study of the reactions of vinyl sulfides (I) with alkyllithium reagents[5] to include cases in which R is vinyl and alkyl. The vinyl sulfides were allowed to react with an excess of alkyllithium in ether at 0°; the compounds studied and the results observed are summarized in Table I.



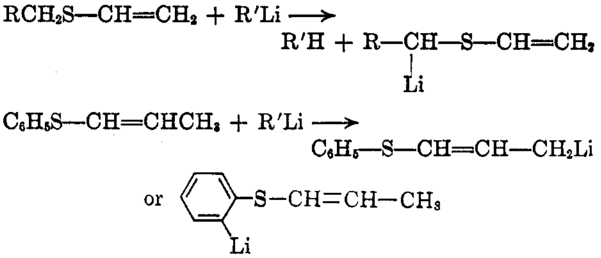
$$\underset{\text{I}}{R{-}S{-}CH{=}CH_2} + R'Li \quad \underset{\text{II}}{R{-}S{-}\underset{Li}{CH}{-}CH_2R'}$$

It can be seen that the addition of RLi to RSCH=CH$_2$ is quite sensitive to the structure of R, and proceeds to a reasonable extent, under the conditions studied, only when R is aromatic or vinyl. The facile addition of alkyllithium to phenyl vinyl sulfide, and the failure of phenyl vinyl ether to react,[5] does indeed suggest a definite sulfur 3-d-orbital effect; however, the reaction of the former may be promoted by the deshielding of the sulfur nucleus through the participation of forms such as III.[6] There is other evidence to support such an activating effect by an aromatic substituent. Considerably more drastic conditions are required for the isomerization of alkyl allyl sulfides[7] than are required for aryl allyl sulfides, and no condensation occurs with benzaldehyde when phenylthioglycolic acid is replaced by alkylthioglycolic acids.[8]



III

Failure of R'Li to react appreciably with the aliphatic vinyl sulfides could logically be attributed to prior anion formation as shown in the accompanying equations

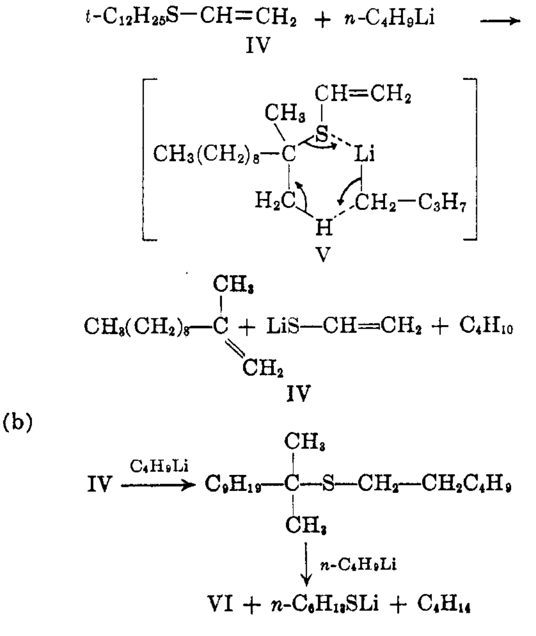


$$RCH_2S{-}CH{=}CH_2 + R'Li \longrightarrow R'H + R{-}\underset{Li}{CH}{-}S{-}CH{=}CH_2$$

$$C_6H_5S{-}CH{=}CHCH_3 + R'Li \longrightarrow C_6H_5{-}S{-}CH{=}CH{-}CH_2Li$$

However, this does not appear to be the case. When the reaction mixture obtained from *n*-butyl vinyl sulfide and ethyllithium was treated directly with carbon dioxide, the olefin was recovered (81%) and there was no evidence for the formation of an organic acid other than propionic. Similarly, and more unexpected, the carbonation of the reaction product obtained from phenyl propenyl sulfide and ethyllithium afforded only recovered sulfide (94.5 %) and propionic acid.

The reaction of *t*-dodecyl vinyl sulfide (IV) with *n*-butyllithium is of interest since cleavage, rather than simple addition resulted. Failure to isolate *n*-hexyl mercaptan from the reaction product suggests that the reaction proceeds as shown in (a), possibly through the complex (V), rather than by path b.



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[1] This work was supported in part by the Office of Ordnance Research, U.S. Army, Contract No. DA-11-002-ORD-2616 and DA-ORD-31-124-61-G13.

[2] In part from the M.S. thesis of M. A. Kalnins, the University of Minnesota, 1959.

[3] In part from the Ph.D. thesis of D. R. Theissen, the University of Minnesota, 1961.

[4] Sinclair Research Fellow, 1959–1961.

[5] W. E. Parham and R. F. Motter, *J. Am. Chem. Soc.*, **81**, 2146 (1959).

[6] The sulfur 3-d-orbital may be made more respectable by interaction with the antibonding benzene orbitals, interactions involving the 3-p-orbitals of sulfur with the benzene π system, and/or by inductive effects.