[CONTRIBUTION FROM THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY, SHREWSBURY, MASS.]

# 19-Labeled Androgens[1]

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Testosterone-19-$C^{14}$ has been prepared by alkylating a readily accessible derivative of 19-nortestosterone with methyl iodide-$C^{14}$. Androst-4-ene-3,17-dione-19-$H^3$ was prepared by ring opening of 5$\beta$,19-cycloandrostane-3,17-dione with a solution of tritiated aqueous hydrochloric acid or aqueous sodium hydroxide. A simple preparation of this cyclopropane derivative is described.

The study of the biological conversion of androgens to estrogens would be very much facilitated by preparing C-19 labeled androgens which, upon aromatization, would yield labeled formaldehyde and/or formic acid. Because of their difficult accessibility, steroids labeled at the angular C-19 methyl group were hitherto unknown.[2] It was therefore felt desirable to synthesize such a labeled compound and the following investigation describes the preparation of testosterone-19-$C^{14}$ and of androst-4-ene-3,17-dione-19-$H^3$.

For the preparation of the former, 19-nortestosterone acetate served as the starting material which was ozonized to its keto acid[3] (**1a**); the acid was then converted by diazomethane to its methyl ester (**1b**). The methyl ester (**1b**) was alkylated with methyl iodide-$C^{14}$ in *t*-butyl alcohol containing potassium *t*-butoxide. Usual work-up gave a crude alkylation product containing **2**, which was not isolated but directly cyclized with acetic anhydride containing anhydrous sodium acetate. The resulting 17$\beta$-acetoxy-4-oxaandrost-5-en-3-one-19-$C^{14}$ [4] (**3**) was obtained in a 1% yield.[5] The reaction[4] of **3** with methylmagnesium iodide, followed by hydrolysis and cyclization, yielded **4** in a yield of 0.5% calculated on methyl iodide-$C^{14}$.

19-Hydroxyandrost-4-ene-3,17-dione[6] (**5**) was used as starting material for the preparation[7] of 19-tritiated androst-4-ene-3,17-dione. Tosylation of **5** with *p*-toluenesulfonyl chloride gave 19-tosyloxandrost-4-ene-3,17-dione (**6**). Its transformation into 5$\beta$,19-cycloandrost-4-ene-3,17-dione could be effected in several ways. The lithium aluminum hydride reduction of **6** in tetrahydrofuran gave a mixture of androst-4-ene-3,17-diol and of 5$\beta$,19-cycloandrostane-3,17-diol (both 3,17-isomeric mixture). These products were not purified but directly oxidized with chromic acid to the known 5$\beta$,19-cycloandrost-4-ene-3,17-dione[8] (**7**) and androst-4-ene-3,17-dione (**8**).

It seems most likely[9] that lithium aluminum hydride reduction would take place on the two carbonyl functions first, followed by hydride attack at C-4, causing displacement of the 19-tosyloxy group with concomitant formation of the cyclopropane ring. This mechanism is supported by the following experiments. The sodium borohydride reduction of 19-tosyloxyandrost-4-ene-3,17-dione (**6**) gave 19-tosyloxyandrost-4-ene-3,17-diol (**9**), which could be converted to 5$\beta$,19-cycloandrostane-3,17-diol by reduction with lithium aluminum hydride in tetrahydrofuran. No transformation took place when the solvolysis of the tosyloxy group was attempted by refluxing **9** for 3 hr. in tetrahydrofuran. The hydride addition at C-4, leading to the formation of the cyclopropane compound, was proved by reducing **6** with lithium aluminum deuteride, followed by oxidation of the crude reaction mixture with chromic acid. Besides androst-4-ene-3,17-dione-*d*, which was not analyzed further, there was obtained 5$\beta$,19-cycloandrostane-3,17-dione-*d*, containing 64%[10] deuterium. Equilibration with base and concomitant rearrangement to androst-4 ene-3,17-dione gave a deuterium-free product, proving thereby the presence of deuterium at position 4.

The cyclopropyl derivative (**7**) was also obtained when, in close analogy to the procedure of Stork and Tsuji,[11] a solution of the tosylate in tetrahydrofuran was treated with lithium in liquid ammonia. Decomposition of the reaction mixture by addition of ammonium chloride, followed by the usual work-up, gave 5$\beta$,19-cycloandrostane-3,17-dione (**7**) in about 20% yield.

Gnoj, *et al.*,[12] have shown that reductive displacement of the halide (A of Fig. 1) takes place with the formation of the 5,9-cyclo compound as shown. Since reduction with zinc, *e.g.*, reduction of $\alpha$-ketoacetates, occurs in a mechanistically analogous manner (the electron pair attacks the oxygen of the carbonyl) formation of the 5$\beta$,19-cyclo compound (B) by the action of zinc and acetic acid on the 19-halo compound could be expected to take place in competition with reduction of the 19-methyl compound. This was indeed found to be the case. When 19-iodoandrost-4-ene-3,17-dione, prepared by treating 19-tosyloxyandrost-4-ene-3,17-dione with sodium iodide in acetone, was treated with zinc and acetic acid, only 5$\beta$,19-

(1) Presented, in part, at the 145th National Meeting of the American Chemical Society, New York, N. Y., September, 1963. This work was supported, in part, by National Institutes of Health Grants A-3419 and AM-07280.

(2) While this paper was being written, there appeared a publication on the introduction of deuterium into C-19 [C. Djerassi and M. A. Kielczewski, *Steroids*, **2**, 125 (1963)] and a note on the labeling of C-19 with $C^{14}$ [P. N. Rao and L. R. Axelrod, *Chem. Ind.* (London), 1838 (1963).

(3) J. A. Hartman, A. J. Tomasewski, and A. S. Dreiding, *J. Am. Chem. Soc.*, **78**, 5662 (1956).

(4) G. I. Fujimoto, *ibid.*, **73**, 1856 (1951).

(5) A smaller excess of methyl iodide-$C^{14}$ (fivefold in this experiment) could increase the poor yield somewhat. However, the n.m.r. spectrum of unpurified **2**, prepared by alkylation under thermodynamic conditions, indicates that the major product is alkylated at C-6. These results should be contrasted with those obtained by M. P. Hartshorn and E. R. H. Jones, *J. Chem. Soc.*, 1312 (1962), who obtained from a closely related compound under acid catalysis a mixture in which the $\Delta^{5(10)}$-isomer prevailed.

(6) A. S. Meyer, *Experientia*, **11**, 99 (1955).

(7) The preparative pathway is marked by bold-typed arrows.

(8) J. J. Bonet, H. Wehrli, and K. Schaffner, *Helv. Chim. Acta*, **45**, 2615 (1962); L. H. Knox, E. Velarde, and A. D. Cross, *J. Am. Chem. Soc.*, **85**, 2533 (1963).

(9) For examples of closely related reaction mechanisms see H. Schmid and P. Karrer, *Helv. Chim. Acta*, 1371 (1949); E. J. Corey, M. G. Howell, A. Boston, R. C. Young, and R. A. Sneen, *J. Am. Chem. Soc.*, **78**, 5036 (1956).

(10) The chromic oxide oxidation might have exchanged some of the incorporated deuterium which would account for this relatively low value.

(11) G. Stork and J. Tsuji, *J. Am. Chem. Soc.*, **83**, 2783 (1961).

(12) O. Gnoj, E. P. Oliveto, C. H. Robinson, and D. H. R. Barton [*Proc. Chem. Soc.*, 207 (1961)] describe a related reaction, whereby a two-electron transfer agent (such as chromous ion) attacks oxygen and thereby transforms 11$\beta$,21-diacetoxy-9$\alpha$-bromo-17$\alpha$-hydroxypregna-1,4-diene-3,20-dione to a 5,9-cyclo structure.

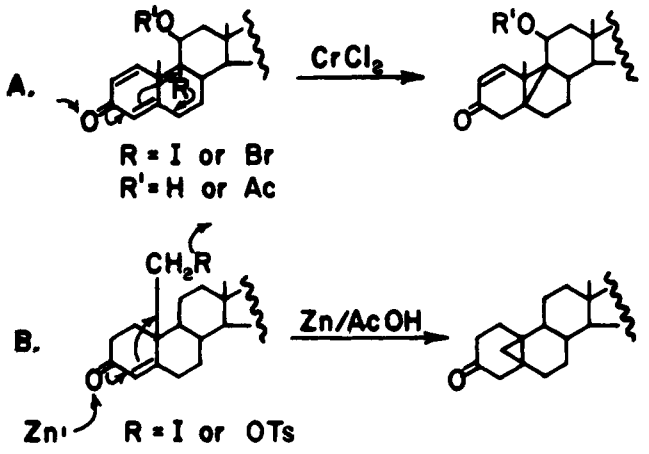



Figure 1.

cycloandrostane-3,17-dione was formed. The same reaction could be extended to 19-tosyloxyandrost-4-ene-3,17-dione.

The opening of the cyclopropane ring took place by boiling **7** in aqueous dioxane to which a trace of either acid or alkali had been added. When the reaction was carried out with tritiated water there was obtained androst-4-ene-3,17-dione-19-H[3].

## Experimental[13]

**Methyl 17β-Acetoxy-3,5-seco-4,19-bisnorandrostan-5-one-3-oate (1b) from 1a.**—To a cold solution of 1.10 g. of 17β-acetoxy-3,5-seco-4,19-bisnorandrostan-5-one-3-oic acid[3] (**1a**) in 15 ml. of dry tetrahydrofuran was added an excess of diazomethane in ether solution and the reaction mixture was then allowed to stand at 22° for 1 hr. After concentration *in vacuo* and crystallization from methanol–ether there was obtained 973 mg. of the methyl ester (**1b**), m.p. 81–82°.

*Anal.* Calcd. for $C_{20}H_{30}O_5$: C, 68.54; H, 8.63. Found: C, 68.62; H, 8.46.

**17β-Acetoxy-3,5-seco-4-oxaandrost-5-en-3-one-19-C[14] (3) from 1b.**—The solution of 350 mg. (1 mmole) of **1b** and 560 mg. of potassium *t*-butoxide in 5 ml. of *t*-butyl alcohol was frozen, the flask connected to the vacuum line, and 5 mmoles of methyl iodide-C[14], containing 1 mc., distilled into it. The flask was then sealed and shaken at 25° for 2 hr. After dilution with water and acidifying with acetic acid to pH 5 the mixture was extracted with methylene chloride. The organic layer was washed with water, dried over sodium sulfate, and evaporated to dryness *in vacuo*. The crude residue, containing **2**, was not purified, but directly lactonized by boiling under nitrogen for 3 hr. in 100 ml. of acetic anhydride containing 10 g. of anhydrous sodium acetate. The volatile components were distilled off *in vacuo* and ethyl acetate was added to the yellow residue. The mixture was then washed with water; the solvent was dried over sodium sulfate and evaporated to dryness *in vacuo*. The residue was chromatographed on a thin layer plate developed with 30% ethyl acetate in benzene. After extracting the appropriate zone with methanol and filtering, the extract was evaporated to dryness. Recrystallization from ether gave 22 mg. (15 μc.) of **3**, m.p. 130–133°, identical in all respects with authentic material.[4]

**Testosterone-19-C[14] (4) from 3.**—This conversion has already been described[4] in detail. In this case 10 mg. (6 μc.) of **4**, m.p. 150–153°, was obtained.

**19-Tosyloxyandrost-4-ene-3,17-dione (6).**—Tosylation was accomplished by allowing 4.6 g. of 19-hydroxyandrost-4-ene-3,17-dione[6] (**5**) to stand overnight at 23° in 50 ml. of pyridine containing 7.2 g. of *p*-toluenesulfonyl chloride. After pouring the mixture on approximately 500 g. of crushed ice and letting it stand for 4 hr., the precipitated solids were filtered off and dried at 50°.

(13) The melting points are corrected. Analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. The mass spectra were recorded by Dr. Ragnar Ryhage, Karolinska Institutet, Stockholm. The nuclear magnetic resonance spectra were obtained in deuteriochloroform solution with a Varian Model V-4300B spectrometer, using tetramethylsilane as an internal standard. Optical rotations were obtained in chloroform solutions.

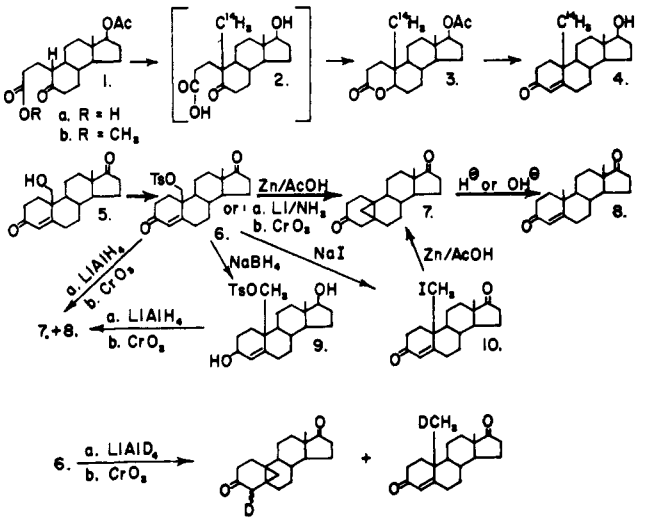






Figure 2.

Refluxing of the crude product in 100 ml. of ether followed by filtration yielded 5.2 g. of **6**, m.p. 162–163° dec., $[\alpha]^{22}D$ +161 ± 2° (*c* 1.2); $\nu_{max}^{KBr}$ 1730 (17-ketone), 1670 (3-ketone), 1620 (C=C of $\Delta^4$-3-ketone), 1600 (aromatic C=C) cm.$^{-1}$.

*Anal.* Calcd. for $C_{26}H_{32}O_5S$: C, 68.39; H, 7.06; S, 7.02. Found: C, 68.45; H, 7.01; S, 7.07.

**5β,19-Cycloandrostane-3,17-dione[8] (7) from 6. A. With Zinc–Acetic Acid.**—A solution of 400 mg. of 19-tosyloxyandrost-4-ene-3,17-dione (**6**) in 100 ml. of 50% aqueous acetic acid was treated under reflux with 5 g. of zinc dust for 90 min. The still hot solution was filtered and the zinc washed with methanol. Removal of the combined solvents *in vacuo* furnished, after recrystallization from acetone–ether, 361 mg. of **7**, m.p. 138–140°, $[\alpha]^{22}D$ +113 ± 2° (*c* 1.0); $\nu_{max}$ 3080 (C–H stretching of cyclopropane), 1730 (17-ketone), 1705 (3-ketone), and 1018 (cyclopropane ring vibration) cm.$^{-1}$.[14,15] The n.m.r. spectrum shows two doublets for the 19-$CH_2$ typical of an AB system with peaks centered at τ 9.43 and 9.53 ($J_{AB}$ = 6 c.p.s.).

*Anal.* Calcd. for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15. Found: C, 79.83; H, 9.10.

**B. With Sodium Iodide, Followed by Zinc–Acetic Acid.**—A solution of 400 mg. of **6** in 10 ml. of acetone and 300 mg. of sodium iodide was heated at 140° in a sealed tube for 3 hr. After cooling, the crystalline precipitate of sodium tosylate (125 mg., 90%) was filtered off. The filtrate was evaporated to dryness and the residue dissolved in ether. The ether solution was washed with water, aqueous sodium thiosulfate solution, 2 *N* sodium carbonate solution, and water, dried over anhydrous sodium sulfate, and evaporated to dryness. The amorphous residue which weighed 351 mg. was reduced with zinc in acetic acid as described above and chromatographed on a silica gel column. From the benzene–ethyl acetate fractions there was obtained, after recrystallization from acetone–ether, 281 mg. of **7**, identical in all respects with the material obtained above.

**C. With Lithium–Ammonia, Followed by Chromic Oxide Oxidation.**—The solution of 200 mg. of 19-tosyloxyandrost-4-ene-3,17-dione (**6**) in 15 ml. of tetrahydrofuran was added at once to a stirred solution of 400 mg. of lithium in 150 ml. of liquid ammonia. After letting the Dry Ice-cooled solution stand for 2 hr., 5 g. of ammonium chloride was added and the ammonia allowed to evaporate. Isolation with methylene chloride afforded 110 mg. of a crude product. Its infrared absorption spectrum showed a strong hydroxyl band at 3500 cm.$^{-1}$ and no carbonyl absorption. This material was dissolved in 5 ml. of absolute acetone and cooled to 0–5°. A solution of chromic acid[16] was added dropwise until the brown color persisted. The mixture was stirred for 5 min. at 0–5° and then for another 5 min. at room temperature. Excess reagent was decomposed by adding 2 ml. of methanol and then the mixture was poured into water. The precipitate was extracted with methylene chloride and the organic layer washed

(14) A. R. H. Cole, *J. Chem. Soc.*, 3807, 3810 (1954).

(15) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," Methuen & Co., Ltd., London, 1957, p. 29.

(16) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

with a 2 *N* solution of sodium hydrogen carbonate and with water and dried over sodium sulfate. Removal of solvent yielded 93 mg. of a crude product, which, after chromatography on a silica gel column and recrystallization from methylene chloride–ether, gave 37 mg. of **7**.

**D. With Lithium Aluminum Hydride, Followed by Chromic Acid Oxidation.**—A solution of 200 mg. of the tosylate (**6**) in 20 ml. of absolute tetrahydrofuran was added at once to a solution of 500 mg. of lithium aluminum hydride in 50 ml. of tetrahydrofuran and the mixture refluxed for 20 hr. After cooling, the excess reagent was decomposed with ethyl acetate, a saturated solution of sodium sulfate and then anhydrous sodium sulfate was added and the precipitated inorganic material filtered off. After thoroughly washing the residue with ethyl acetate, the combined solutions were dried over sodium sulfate and evaporated *in vacuo* to yield 195 mg. of a crystalline alcohol, m.p. 131–145°, which showed in the infrared absorption spectrum a large hydroxyl band and no bands for aromatic C=C or carbonyl groups. The crude product was oxidized with chromic acid under the same conditions as described. Chromatographic separation of the mixture on a column of silica gel furnished 23 mg. of **7**, identical in all respects with authentic material. No androst-4-ene-3,17-dione (**8**) could be isolated.

**E. With Sodium Borohydride Followed by Lithium Aluminum Hydride.**—To a solution of 200 mg. of **8** in 50 ml. of ethanol was added a solution of 200 mg. of sodium borohydride in 50 ml. of ethanol; it was allowed to stand for 18 hr. at 25°. After pouring the solution on crushed ice and letting the mixture warm up to room temperature, the solids were filtered off and dried. The crude tosylate showed infrared absorption maxima at 3500 (—OH), 1580, and 1615 (aromatic C=C) cm.$^{-1}$.

The crude sodium borohydride reduction product was now reduced with lithium aluminum hydride and the reaction product oxidized with chromic acid as indicated above, whereby 19 mg. of **7** and 17 mg. of **8** could be isolated.

**5β,19-Cycloandrostane-3,17-dione-4-*d* and Androst-4-ene-3,17-dione-19-*d* from 6.**—19-Tosyloxyandrost-4-ene-3,17-dione was first reduced with lithium aluminum deuteride and then the crude mixture was oxidized with chromic acid using the same conditions as those employed before. Separation on a thin layer chromatographic plate, using silica gel as adsorbent and 30% ethyl acetate in benzene as eluent, furnished a 1:1 mixture of androst-4-ene-3,17-dione-19-*d* and 5β,19-cycloandrostane-3,17-dione-4-*d*, $\nu_{max}^{CHCl_3}$ 2142 (C-*d* axial) ad 2167 cm.$^{-1}$ (C-*d* equatorial). The mass spectral analysis of the latter gave

| | | | |
|---|---|---|---|
| *m/e* | 286 | 25% | $d_0$ |
| *m/e* | 287 | 64% | $d_1$ |
| *m/e* | 288 | 54% | $d_2$ |
| *m/e* | 289 | 51% | $d_3$ |

Equilibration with a 2.5% methanolic sodium hydroxide solution followed by isolation of androst-4-ene-3,17-dione gave a product which did not contain any deuterium.

**Androst-4-ene-3,17-dione-19-H$^3$ from 7.**—To the solution of 27 mg. of **7** in 0.5 ml. of dioxane was added by distillation 4 mg. of tritiated water (13 c.) and 0.05 ml. of concentrated hydrochloric acid. After sealing the flask, the solution was heated on a steam bath for 24 hr. and then kept an additional 24 hr. at 25°. The break seal of the flask was then opened, the solvents distilled off, and the residue equilibrated with 2.5% potassium hydroxide in methanol–water (1:2 v./v.).

After addition of water the mixture was extracted with methylene chloride, the extract dried over anhydrous sodium sulfate, and evaporated. The crude mixture was finally chromatographed on a Celite partition column using the Bush A system, which yielded 11 mg. of androst-4-ene-3,17-dione-19-H$^3$ with a specific activity of 20 μc./μg. The radiochemical purity was tested by chromatographing about 1 μc. of the material with 5 μg. of authentic androst-4-ene-3,17-dione on paper on the Bush A system. Scanning in a Vanguard automatic windowless flow chromatogram scanner (Model 800 autoscanner) gave a single sharp peak corresponding to the marked spot due to the carrier and found by inspection with the ultraviolet lamp.

In a second experiment, 20 mg. of **7** was dissolved in a flask containing 1 ml. of dioxane and 0.1 mg. of sodium hydroxide. The flask was connected to the vacuum line, degassed, and evacuated, and 4 mg. of tritiated water (13 c.) was distilled into it. Then the flask was sealed off, removed from the line, heated on a steam bath for 6 hr., and left at room temperature for 18 hr. After opening the break seal, the solvents were distilled off on the vacuum line, and water was added to the residue and worked up exactly as described above. After chromatography, 12 mg. of androst-4-ene-3,17-dione-19-H$^3$ with a specific activity of 30 mc./mg. was isolated.

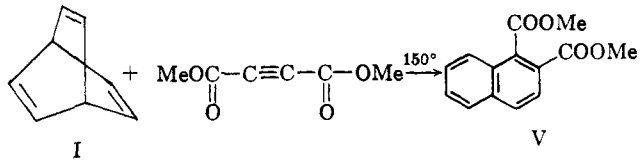
**Acknowledgment.**—The authors thank Dr. Karl Heusler of Ciba Ltd., Basel, Switzerland, for a generous gift of 19-hydroxyandrost-4-ene-3,17-dione and Mr. Thomas Wittstruck for the recording, calculation, and interpretation of the n.m.r. spectra. The methyl 17β-acetoxy-3,5-seco-4,19-bisnorandrostan-5-one-3-oate was first prepared in this laboratory by Dr. H. J. Brodie.

# COMMUNICATIONS TO THE EDITOR

## The Chemistry of Barrelene. II.[1] A Remarkable Transformation to Naphthalene Derivatives

*Sir:*

Following our reported synthesis of barrelene[1,2] (I), we have pursued investigations of the chemistry of this unusual compound. In one of these investigations the remarkable observation was made that barrelene and dimethyl acetylenedicarboxylate when heated to 150° afforded dimethyl naphthalene-1,2-dicarboxylate (V) in 12% yield.




I V

(1) For Part I, *cf.* H. E. Zimmerman and R. M. Paufler, *J. Am. Chem. Soc.*, **82**, 1514 (1960).

(2) The trivial name of "barrelene" was suggested for bicyclo[2.2.2]-octa-2,5,7-triene (I) because of the barrel shaped array of molecular orbitals possessed by this six electron π-system. However, electronic interaction and electron delocalization enforced by orbitals held within distance for appreciable overlap does not imply whether this interaction is stabilizing, destabilizing, or neither [*e.g.*, bringing the terminal *p*-orbitals of hexatriene within overlap distance leads to stabilizing interaction (benzenoid electronics), bringing the terminal p-orbitals of butadiene within overlap distance leads to loss of delocalization energy (cyclobutadiene electronics), bringing three ethylenic moieties together with barrelene geometry leads to no change in delocalization energy]. The point that barrelene has no delocalization energy was clearly stated (ref. 1) but clarification of this seemingly obvious distinction between interaction and stabilizing interaction appears necessary because we have been misquoted [J. M. Tedder, *Ann. Rept. Prog. Chem.*, **62**, 232 (1960)] as suggesting barrelene to be stabilized and then criticized for this opinion opposite to our published statement. Still another criticism seemed to be voiced by Tedder. But since we do not know how to interpret the comment "They even go so far as to suggest that the compound should be called barrelene," we are unable to reply to this. In contrast, one should note the more perceptive review of our work by M. F. Ansell in the same volume [*ibid.*, **62**, 246 (1960)]; unfortunately, Ansell's accurate report was not indexed.