[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, CIBA PHARMACEUTICAL PRODUCTS, INC., SUMMIT, N. J., AND THE INSTRUMENT DIVISION, VARIAN ASSOCIATES, PALO ALTO, CALIF.]

Rauwolfia Alkaloids. XLI. Methyl Neoreserpate, an Isomer of Methyl Reserpate. Part 3. Conformations and N.m.r. Spectra

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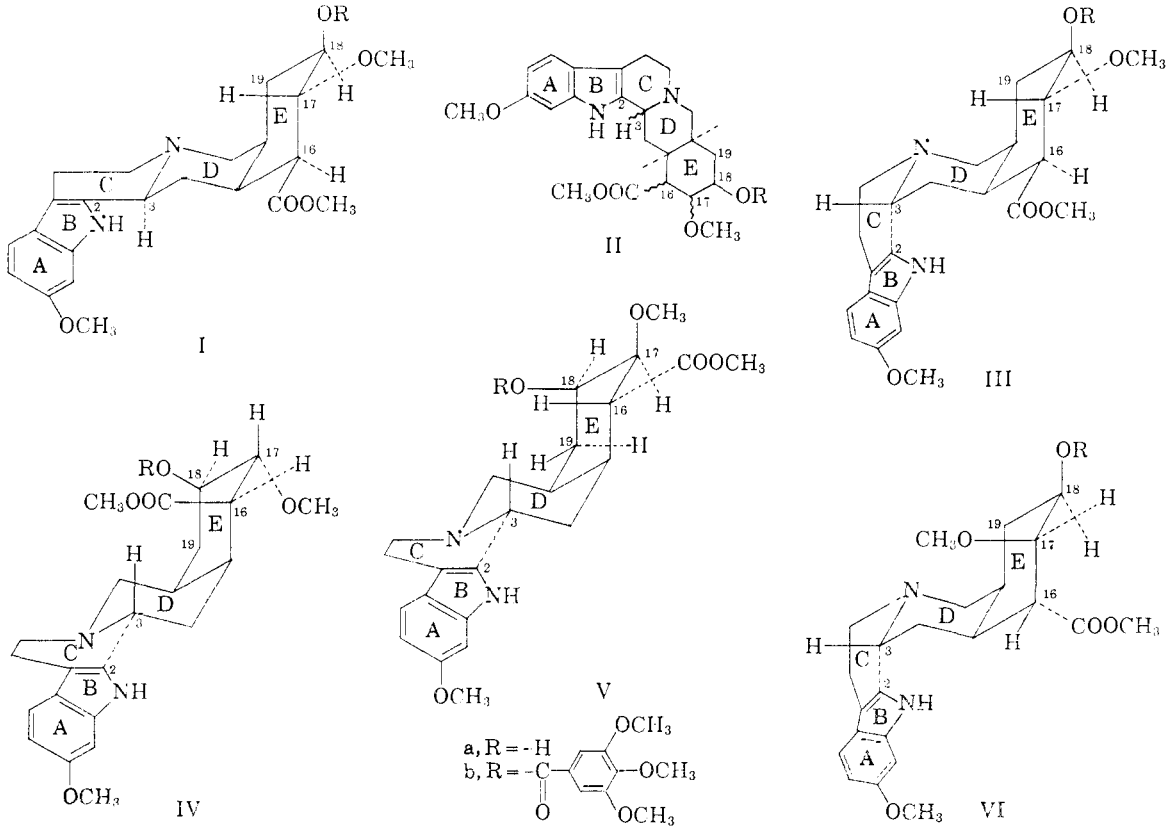
RECEIVED JULY 5, 1961

The nuclear magnetic resonance spectra of methyl 3-isoreserpate, methyl reserpate, methyl neoreserpate, and the three corresponding 3′,4′,5′-trimethoxybenzoate esters provide strong support for the assignment of conformations Ia, IIIa and Va to the alcohols, and Ib, IIIb and Vb to the esters.

In our previous papers[1-3] we used the generally-accepted[4] conformation of methyl reserpate (IIIa) and the assumed conformation of methyl neoreserpate (Va) to explain the formation of methyl neoreserpate from methyl reserpate, to rationalize differences in rates of equilibration of methyl reserpate and methyl neoreserpate with a hypothetical intermediate, and to modify a correlation of infrared spectra with configuration at C-3. Although conformations IIIa and Va are consistent with all the known facts, they are actually assumptions based mainly on analogy and on chemical intuition. It seemed important to us to obtain independent support for these conformations. We have therefore examined the nuclear magnetic resonance (n.m.r.) spectra of methyl 3-isoreserpate, methyl reserpate, methyl neoreserpate, and the three corresponding 3′,4′,5′-trimethoxybenzoate esters. Our results offer strong support for the accuracy of I, III and V as the predominant conformations.



The structural formula for all six compounds is represented by II, where R is hydrogen for the alcohols and trimethoxybenzoyl for the esters. Methyl 3-isoreserpate differs from methyl reserpate only at C-3,[4] whereas methyl neoreserpate differs from methyl reserpate only at C-16 and C-17.[1] Methyl 3-isoreserpate must have structure Ia; the alternate all-chair structure would be much less stable because large functional groups would be axial at C-3, C-16, C-17 and C-18. Methyl reserpate differs from methyl 3-isoreserpate only at C-3, and in its stable conformation (IIIa) would be

(1) W. E. Rosen and J. M. O'Connor, *J. Org. Chem.*, **26**, 3051 (1961).
(2) W. E. Rosen and H. Sheppard, *J. Am. Chem. Soc.*, **83**, 4240 (1961).
(3) W. E. Rosen, *Tetrahedron Letters*, No. 14, 481 (1961).
(4) For leading references, see (a) E. Schlittler in R. E. Woodson, Jr., H. W. Youngken, E. Schlittler and J. A. Schneider, "Rauwolfia: Botany, Pharmacognosy, Chemistry, and Pharmacology," Little, Brown and Co., Boston, Mass., 1957, pp. 74–96. (b) P. E. Aldrich, P. A. Diassi, D. F. Dickel, C. M. Dylion, P. D. Hance, C. F. Huebner, B. Korzun, M. E. Kuehne, L. H. Liu, H. B. MacPhillamy, E. W. Robb, D. K. Roychaudhuri, E. Schlittler, A. F. St. André, E. E. van Tamelen, F. L. Weisenborn, E. Wenkert and O. Wintersteiner, *J. Am. Chem. Soc.*, **81**, 2481 (1959).

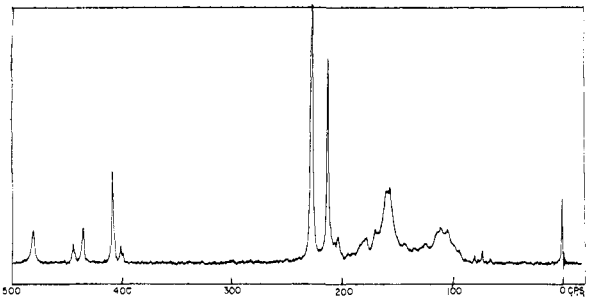


Fig. 1.—Nuclear magnetic resonance spectrum of methyl 3-isoreserpate (Ia).

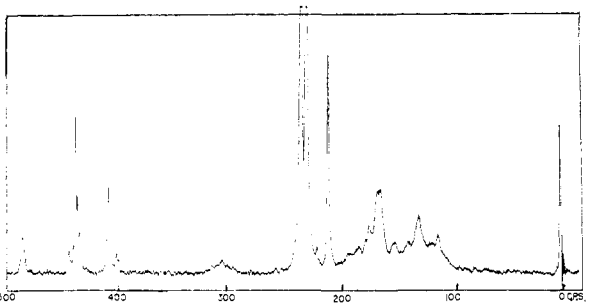


Fig. 2.—Nuclear magnetic resonance spectrum of methyl 3-isoreserpate 3′,4′,5′-trimethoxybenzoate (Ib).

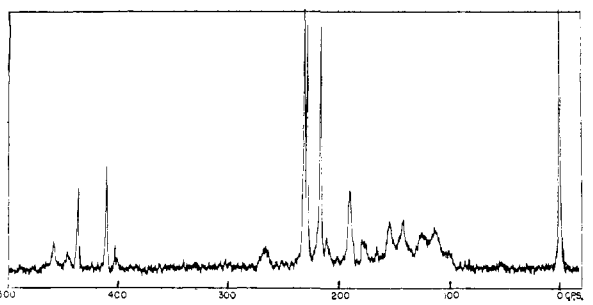


Fig. 3.—Nuclear magnetic resonance spectrum of methyl reserpate (IIIa).

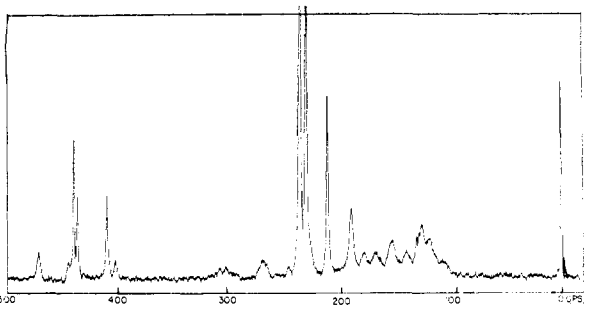


Fig. 4.—Nuclear magnetic resonance spectrum of methyl reserpate 3′,4′,5′-trimethoxybenzoate (reserpine) (IIIb).

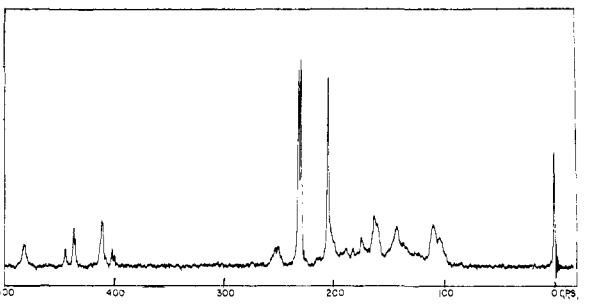


Fig. 5.—Nuclear magnetic resonance spectrum of methyl neoreserpate (Va).

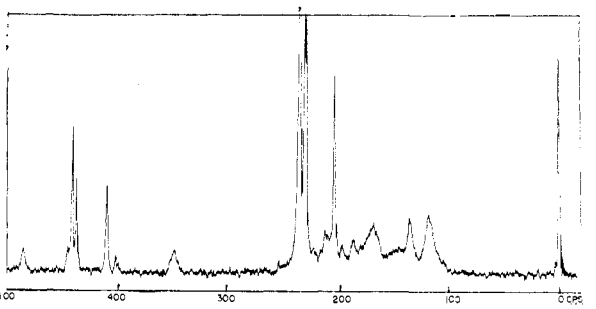


Fig. 6.—Nuclear magnetic resonance spectrum of methyl neoreserpate 3′,4′,5′-trimethoxybenzoate (Vb).

expected to have an equatorial hydrogen (with respect to ring D) at C-3, and axial hydrogens at C-16, C-17 and C-18. In its less stable conformation (IVa), from which some derivatives form (*e.g.*, reserpic acid lactone),[4] methyl reserpate would have an axial hydrogen at C-3 and equatorial hydrogens at C-16, C-17 and C-18. Methyl neoreserpate has been assumed[1-3] to have structure Va, which is analogous in C/D/E ring conformation to the *less* stable conformation of methyl reserpate (IVa). The hydrogens of Va are axial at C-3, C-16 and C-17, and equatorial at C-18. The conformation of methyl neoreserpate which has been assumed to be less stable (VIa) would have equatorial hydrogens at C-3 (with respect to ring D), C-16 and C-17, and an axial hydrogen at C-18.

The n.m.r. spectra[5] of the compounds studied are shown in Figs. 1–6. The spectra of the three alcohols, Figs. 1, 3 and 5, show the peak for the proton on the indole nitrogen beyond 450 c.p.s. from the reference. The three compounds show similar patterns for the three aromatic (ring A) protons between 400 and 450 c.p.s. The protons of the aromatic methoxyl are probably represented in all three spectra by the peak at 228 c.p.s., those of the carbomethoxyl group by the peak at 228, 231 or 232 c.p.s. (Figs. 1, 3 and 5, respectively), and those of the C-17 methyl ether by the peak at 212, 216 or 205 c.p.s. (respectively).

Methyl 3-isoreserpate has been assumed[4] to have structure Ia. Since axial protons are generally observed to lie at higher fields (lower c.p.s.) than corresponding equatorial protons, it is not surprising that in the spectrum of methyl 3-isoreserpate (Fig. 1), no signals attributable to protons on saturated carbon atoms are located to the left (lower fields) of the methoxyl peaks.

The methyl reserpate spectrum (Fig. 3) shows a signal at 266 c.p.s. If structure IIIa correctly represents methyl reserpate, the main change from Ia is the change from an axial proton at C-3 to an

(5) The spectra were obtained with the Varian A-60 spectrometer at 60 mc./sec. using deuterated chloroform as solvent and tetramethylsilane as internal reference. The tetramethylsilane peak falls at 0 on the right of each curve. The sweep width of 500 c.p.s. was covered in a sweep time of 250 seconds. Chemical shifts are quoted in c.p.s. and can be converted to field-independent δ units (p.p.m.) by dividing by 60, where δ is defined by the relation $\delta = 10^6(H_{ref} - H)/H_{ref}$.

equatorial proton, and the peak at 266 c.p.s. represents that equatorial C-3 proton.[6] If IVa had correctly represented methyl reserpate, two or three of the equatorial protons (at C-16, C-17 and C-18) might be expected to have shifted downfield into the region to the left of the methoxyl peaks.

The methyl neoreserpate spectrum (Fig. 5) shows a peak at 253 c.p.s., which has a quartet structure with intensities 1-3-3-1. This quartet structure is not well-defined in the spectrum shown in Fig. 5, but shows up clearly in a spectrum taken on the high resolution Varian research model HR-60 spectrometer. The 1-3-3-1 intensity distribution requires that the observed proton have *three* proton neighbors, all with approximately equal spin couplings. This is exactly what would be expected for the C-18 proton of Va, since equatorial protons are coupled about equally strongly to axial or to equatorial neighbors. Furthermore, the observed coupling constant of 2–3 c.p.s. is consistent with that expected for a proton in such an environment. No other proton of methyl neoreserpate meets these requirements. If the assignment of the 266 c.p.s. peak of the methyl reserpate spectrum was correctly assigned to its equatorial C-3 proton, then the absence of a peak near 266 c.p.s. in the spectrum of methyl neoreserpate means that its C-3 proton must be axial.

The carbomethoxyl groups of Ia, IIIa and Va are equatorial, and all have a neighboring equatorial methoxyl group. Consequently, no large variations would be expected in the position of the peak representing the protons of the carbomethoxyl group in the three compounds, and no significant variations are found. The methoxyl group at C-17 is equatorial in all three compounds, but it is surrounded by equatorial substituents (carbomethoxyl and hydroxyl) in Ia and IIIa and by one equatorial and one axial substituent in Va. It is not surprising, therefore, that the methyl ether peak falls at 212 c.p.s. and 216 c.p.s. for Ia and IIIa, respectively, but shifts slightly to 205 c.p.s. in Va.

Acylation of a hydroxyl group is known to cause a downfield shift (increased c.p.s.) of the peak for the proton on the carbon holding the hydroxyl.[7] If the assignments described above are correct, acylation of the C-18 hydroxyl (IIa → IIb) should result in a large shift of the 253 c.p.s. peak of Va to higher c.p.s. but in no shift of the 266 c.p.s. peak of IIIa. In addition, the signal for the axial C-18 proton of methyl 3-isoreserpate and of methyl reserpate might be expected to shift far enough downfield after acylation to be observed to the left of the methoxyl peaks. The n.m.r. spectra of the trimethoxybenzoate esters of methyl 3-isoreserpate, methyl reserpate and methyl neoreserpate are shown in Figs. 2, 4, and 6, respectively. The spectra confirm and extend the interpretations made on the basis of the spectra of the parent alcohols.

The spectrum of methyl 3-isoreserpate trimethoxybenzoate (Fig. 2) shows a rather broad peak centered at about 305 c.p.s., in contrast to the spectrum of methyl 3-isoreserpate itself (Fig. 1), which shows no peak in this region. The broad 305 c.p.s. peak can be attributed to the shifted axial C-18 proton with its two axial and one equatorial neighbors. Since axial–axial coupling constants are usually about 9 c.p.s. and axial–equatorial coupling constants are 2–3 c.p.s., the C-18 proton peak is split first into a triplet with 1-2-1 intensity ratios and spaced about 9 c.p.s. apart, and then each line of the triplet is doubled by the smaller axial-equatorial coupling. These couplings account for the breadth of the 305 c.p.s. peak. The spectrum of Ib, therefore, confirms the axial C-18 proton which could be assigned to Ia only on the basis of the absence of peaks in this region.

The spectrum of methyl reserpate trimethoxybenzoate (reserpine) (Fig. 4) shows the same broad peak at about 305 c.p.s. as was found in the spectrum of Ib (Fig. 2), confirming the presence of the axial C-18 proton of IIIa and IIIb. The signal at 266 c.p.s., which was assigned to the equatorial C-3 proton in the spectrum of IIIa, remains unchanged in the spectrum of IIIb, as expected.

Finally, the spectrum of methyl neoreserpate trimethoxybenzoate (Fig. 6) shows a downfield shift of the 253 c.p.s. peak to 347 c.p.s. This shift confirms the original assignment of the 253 c.p.s. peak to the equatorial C-18 proton of Va. The quartet structure of this peak is not evident from the spectrum shown in Fig. 6, but a trace of Vb taken with the Varian research model HR-60 spectrometer shows the quartet very clearly. The quartet structure of the peak with its 1-3-3-1 intensity distribution confirms its assignment to an equatorial proton having small and equal spin coupling to three neighboring protons.

Study of the n.m.r. spectra, therefore, confirms conformations Ia, IIIa and Va for methyl 3-isoreserpate, methyl reserpate and methyl neoreserpate, respectively, and conformations Ib, IIIb and Vb for their corresponding trimethoxybenzoate esters. Other conformations of these compounds, which are in equilibrium with conformations I, III and V, are evidently present only in small amounts. The results also suggest that n.m.r. spectroscopy might prove useful in the study of related alkaloids whose structures are not yet firmly established.

Experimental[8]

The alkaloids used in these studies were homogeneous by paper chromatography and had the following physical properties.

Methyl 3-isoreserpate,[9] m.p. 222–224°, $[\alpha]_D$ −58.8°; previously reported[10]: m.p. 220–221°; $[\alpha]_D$ −62° (EtOH).

(6) The lack of resolved fine structure in this peak may be due to spin coupling to the nitrogen, which often smears the peak due to quadrupole relaxation of the nitrogen.

(7) This effect has been observed in a wide variety of compounds. The most closely analogous compounds studied as a class are the steroids. See J. N. Shoolery and M. T. Rogers, *J. Am. Chem. Soc.*, **80**, 5123 (1958).

(8) Melting points were determined in an electrically heated aluminum block using open capillaries, and are uncorrected. Optical rotations were taken in chloroform solution at 25 ± 2° unless otherwise specified. Ultraviolet absorption spectra were determined in ethanol. We thank Mr. L. Dorfman and his associates for the microanalytical data.

(9) Kindly supplied by Dr. R. A. Lucas of the CIBA laboratories.

Anal. Calcd. for $C_{23}H_{39}N_2O_5$ (414.51): C, 66.65; H, 7.30; N, 6.76. Found: C, 66.55; H, 7.54; N, 6.73.

Methyl 3-isoreserpate 3′,4′,5′-trimethoxybenzoate,[9] m.p. 151–153°, $[\alpha]_D$ −164.0°; λ_{max} 215–217 mμ (ε 59,000), 224–230 (sh., 43,800), 265–269 (15,700), 291–295 (9,750), 327–333 (plateau, 330), 363–375 (117); previously reported[10]: m.p. 150–155°, $[\alpha]_D$ −164°. *Anal.* Calcd. for $C_{33}H_{40}N_2O_9 \cdot {}^1/_2CH_3OH$ (624.21): C, 64.41; H, 6.78; N, 4.48. Found: C, 64.27; H, 6.50; N, 4.63.

Methyl reserpate, m.p. 243–244.5°, $[\alpha]_D$ −99.5°; previously reported[11]: m.p. 235–240°, $[\alpha]_D$ −106°. *Anal.* Found: C, 66.38; H, 7.34; N, 6.63.

(10) H. B. MacPhillamy, C. F. Huebner, E. Schlittler, A. F. St. André and P. R. Ulshafer, *J. Am. Chem. Soc.*, **77**, 4335 (1955).

Methyl reserpate 3′,4′,5′-trimethoxybenzoate (reserpine), m.p. 267–268°, $[\alpha]_D$ −119.3°; previously reported[11]: m.p. 277–277.5° (vacuum), $[\alpha]_D$ −118°. λ_{max} 216–218 mμ (ε 58,500), 226–228 (sh., 43,200), 266–268 (16,300), 292–296 (10,100). *Anal.* Calcd. for $C_{33}H_{40}N_2O_9$ (608.58): C, 65.12; H, 6.62; N, 4.60. Found: C, 65.54; H, 6.63; N, 4.99.

Methyl Neoreserpate and Methyl Neoreserpate 3′,4′,5′-trimethoxybenzoate.—The physical properties of these samples were previously described.[1] The methyl neoreserpate was anhydrous, and the methyl neoreserpate trimethoxybenzoate contained a half mole of water.

(11) L. Dorfman, A. Furlenmeier, C. F. Huebner, R. Lucas, H. B. MacPhillamy, J. M. Mueller, E. Schlittler, R. Schwyzer and A. F. St. André, *Helv. Chim. Acta*, **37**, 59 (1954).

[CONTRIBUTION FROM THE ENTOMOLOGY RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, BELTSVILLE, MD.]

Insect Sex Attractants. I. The Isolation, Identification, and Synthesis of the Sex Attractant of the Gypsy Moth[1]

BY MARTIN JACOBSON, MORTON BEROZA AND WILLIAM A. JONES

RECEIVED JULY 24, 1961

The extremely potent sex attractant secreted by the female gypsy moth (*Porthetria dispar* (L.)) to lure the male has been isolated in pure form and characterized as dextrorotatory 10-acetoxy-*cis*-7-hexadecen-1-ol. A second, as yet unidentified component, has also been isolated and shows attraction of a lower order. The optically inactive form of the major attractant has been synthesized and found to be as attractive as the natural isomer.

The gypsy moth (*Porthetria dispar* (L.) is one of the most serious pests of fruit, shade and woodland trees in New England and eastern New York State. If left uncontrolled, the insect would threaten hardwood forests from northern Maine to the Ozark Mountains. Based on a 20-year study, losses caused by defoliation have been estimated to run in the tens of millions of dollars.

The female gypsy moth does not fly and the male is attracted to the female by scent.[2] As early as 1913 it was determined that an extract prepared from the last two abdominal segments of virgin females could be used in traps as a powerful male attractant, and a benzene extract of these tips has been used for many years in U. S. Department of Agriculture survey traps.[3] Although an effective lure is a practical necessity in a gypsy moth control or eradication program, the collection of female pupae and the clipping and processing of tips from the emerged adults are time-consuming and costly. Investigations had therefore been under way for some time to isolate and identify the attractant with a view toward possible synthesis of this or a related active compound.[4]

The pioneering detailed chemical studies on the problem were conducted by Haller and Acree,[4,5] who soon determined that the active material was a lipid residing in the unsaponifiable neutral fraction of the extract. It was partially volatile with steam, reacted with phthalic anhydride, and could be recovered from the phthalic acid ester upon saponification. Considerable concentration of the attractant was obtained by chromatography of the neutral fraction in sequence on columns of magnesium carbonate and magnesium oxide.[5c] These fractionations gave definite indications of the presence of more than one substance attractive to males. From the abdominal glands of 100,000 female moths, Acree obtained about 12 milligrams of an active impure fraction which he designated "gyptol." Saponification of "gyptol" with potassium hydroxide in diethylene glycol at 120–130° gave a few milligrams of a solid acid, which has since been identified as palmitic acid,[6] and an unidentified alcohol fraction. Lack of a suitable bioassay method made it necessary for Acree to confine the testing of chemical fractions to the 2–3-week period of natural flight each summer.

A benzene extract of the abdominal tips of 200,000 virgin female gypsy moths was made available for our investigations in 1956, and the extract of an additional 300,000 females was prepared in 1958; these were obtained from pupae collected in Connecticut and in Spain, respectively. In the process of isolating the unsaponifiable fraction, large quantities of free and esterified fatty acids unattractive to male moths[7] were obtained. Butenandt,[8] who attempted to isolate and identify the sex attractant of the silkworm moth (*Bombyx mori* (*L.*)), reported

(1) Reported in part as a note in *Science*, **132**, 1011 (1960).

(2) E. H. Forbush and C. H. Fernald, "The Gypsy Moth, *Porthetria dispar* (Linn.)," Massachusetts State Board of Agriculture, Boston, Mass., 1896, p. 345.

(3) R. F. Holbrook, M. Beroza and E. D. Burgess, *J. Econ. Entomol.*, **53**, 751 (1960).

(4) See H. L. Haller, F. Acree, Jr., and S. F. Potts, *J. Am. Chem. Soc.*, **66**, 1659 (1944), and references cited therein for a history of the problem and the early chemical studies.

(5) (a) F. Acree, Jr., *J. Econ. Entomol.*, **46**, 313 (1953); (b) **46**, 900 (1953); (c) **47**, 321 (1954).

(6) M. Jacobson and M. Beroza, unpublished investigations.

(7) Laboratory bioassay tests were carried out by an unpublished modification of the method of B. C. Block, *J. Econ. Entomol.*, **53**, 172 (1960). Field tests were carried out as described by J. M. Corliss, "U. S. Dept. Agr. Yearbook," Govt. Printing Office, Washington, D. C., 1952, p. 694. The assistance of E. C. Paszek, U. S. Department of Agriculture, Nashua, N. H., in carrying out these tests is gratefully acknowledged.

(8) A. Butenandt, *Nova Acta Leopoldina*, **17**, 445 (1955).