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STRUCTURE AND STEREOCHEMISTRY OF THE MONOBROMOTIGOGENINS[1,2]

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Abstract—Bromination of desoxytigogenin has been found to yield two isomeric 23-bromo-5α-25R-spirostans. The corresponding 3β-acetoxy-23-bromo-5α-25R-spirostans were also prepared from tigogenin acetate. A combination of physical methods based on mass spectrometry and NMR measurements, at 60- and 100-mc/s, have been used to confirm attack by bromine at C-23 of the spiroketal system. The configuration of each epimeric pair (at C-23, cf. III and IV) of isomers was also determined.

EARLY in their study of the steroidal sapogenin spiroketal system Marker and Rohrmann[5] found that sarsasapogenin would yield a monobromo derivative. The product was easily reconverted into sarsasapogenin by reduction with sodium and alcohol. Marker considered C-20 and C-23 as two possible sites for the bromine, since both are adjacent to a potential carbonyl group at C-22 (see I). When a subsequent study involving chromic acid oxidation of bromosarsasapogenin acetate yielded 3β-acetoxy-16-oxo-5α-pregnane 20S-carboxylic acid, Marker eliminated C-20 leaving C-23 as the possible position of the bromine atom.[6] No further evidence for this site was provided and in spite of numerous subsequent investigations, the C-23 bromo structure was never fully established. Djerassi *et al.*[7] reinvestigated Marker's report of the monobromination reaction and showed that dibromo compounds could also be obtained in the 25L series.[8] Somewhat later, Mueller and Norton[9] characterized the isomeric mono- and dibromohecogenin acetates. In the

[1] Part XXIII of series, *Steroids and Related Natural Products*. For the preceding contribution see, G. R. Pettit, D. S. Alkalay, P. Hofer and P. A. Whitehouse, *Tetrahedron* **20**, 1755 (1964).

[2] Financial support for the investigations carried out at the University of British Columbia was provided by the National Research Council of Canada, while the University of Maine program was supported in part by PHS Research Grants CA-04074-04 to CA-04074-06 from the National Cancer Institute, Public Health Service.

[3] Inquiries may be addressed to this author.

[4] Smith Kline and French Postdoctoral Fellow, 1962.

[5] R. E. Marker and E. Rohrmann, *J. Amer. Chem. Soc.* **61**, 846 (1939).

[6] R. E. Marker, D. L. Turner, A. C. Shabica and P. R. Ulshafer, *J. Amer. Chem. Soc.* **63**, 1032 (1941).

[7] C. Djerassi, H. Martinez and G. Rosenkranz, *J. Org. Chem.* **16**, 303 (1951).

[8] Recommendations for the systematic naming of steroidal sapogenins have recently been prepared by G. P. Mueller and G. R. Pettit, *Experientia* **18**, 404 (1962). See also, G. R. Pettit, *Ibid.* **19**, 124 (1963). In regard to the stereochemistry at C-25 a recent communication by E. O'Donnell and M. F. C. Ladd, *Chem. & Ind.* 1984 (1963) is of interest.

[9] G. P. Mueller and L. L. Norton, *J. Amer. Chem. Soc.* **76**, 749 (1954).

absence of any direct evidence for position or configuration, the monobrominated isomers were arbitrarily designated as 23a and 23b isomers.[9] Dickson,[10] Barton *et al.*[11] next studied IR absorption spectra exhibited by the isomeric bromosapogenins and concluded, from stretching frequencies of the C-Br bond, that the 23a- and 23b-bromides are equatorial and axial isomers respectively. During the same period Wall and Jones[12] made a detailed study of the diosgenin and tigogenin side-chain bromination products and again implicated C-23 as the reaction site. However, Wall emphasized that although the C-23 assignment is quite logical, "it is not based on a rigid structure proof." Finally, the bromination reaction has been employed, in a number of instances, to protect the spiroketal system.[13]

After Woodward *et al.*[14] proposed a mechanism involving rupture of the spiroketal system and eventual generation of a potential aldehyde at C-26 (e.g., II) to account for acid-catalyzed interconversion of normal and isosteroidal sapogenins, it became of interest to consider the possibility that such an intermediate might also be generated during bromination: a reaction normally conducted under acidic conditions. Although the reaction requirements for bromination are considerably milder, the presence of an aldehyde intermediate would suggest introduction of bromine at C-25 and subsequent cyclization[14] of the bromoaldehyde could then yield the corresponding 25-bromosapogenin. In order to determine whether such a process was in fact operative, we undertook a study directed at elucidating the structure of a typical series of bromosapogenins. For this purpose, 5α-25R-spirostan (I, desoxytigogenin)[7] and 3β-acetoxy-5α-25R-spirostan (tigogenin acetate) were selected.

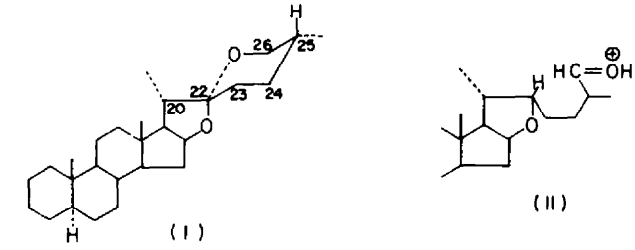
Bromination[7] of desoxytigogenin was found to afford two monobrominated products: one (IIIa) melting at 193–194°, $[\alpha]_D^{22}$ −64·3°, and a previously unreported isomer (IVa) melting at 215–217° to 225–226° (depending on rate of heating), $[\alpha]_D^{22}$ −87·4°. In each case, the results of both elemental and mass spectral (Fig. 1) analyses eliminated the possibility of incomplete or excessive bromination. The mass spectral fragmentation patterns of both isomers between the molecular ion (480) and m/e 257 were essentially the same. The mass spectrum of both isomers displayed a peak at m/e 331 which has been assigned[15] ion V. Similarly a peak at m/e 331 appears in the mass spectrum of desoxytigogenin.[15] A bromine substituent at C-20 in either of the isomeric monobromodesoxytigogenins was, therefore, excluded and this conclusion was substantiated by results of a NMR study (*vide infra*).

When tigogenin acetate was brominated as previously described[12] two isomeric bromotigogenin acetates were obtained: IIIb, m.p. 206–208°, $[\alpha]_D^{22}$ −61·9° (*c*, 1·19) and IVb, m.p. 201–202°, $[\alpha]_D^{22}$ −57·0° (*c*, 1·28). Elemental analyses for bromine confirmed that both isomers were the products of monobromination.

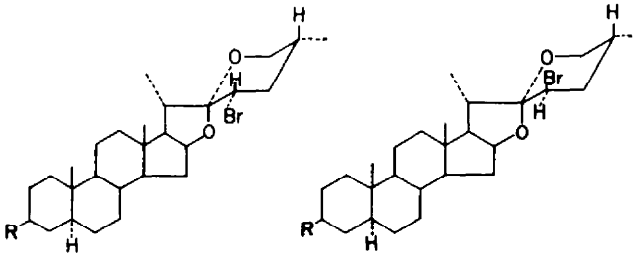
At an early stage in our investigation of the bromotigogenins (III and IV), it appeared that NMR spectroscopy could provide the required structural information. For this reason, a detailed NMR investigation of representative steroidal sapogenins in the 25D and 25L series was initiated and some generalizations arising from this

[10] D. H. W. Dickson and J. E. Page, *J. Chem. Soc.* 447 (1955).
[11] D. H. R. Barton, J. E. Page and C. W. Shoppee, *J. Chem. Soc.* 331 (1956).
[12] M. E. Wall and H. W. Jones, *J. Amer. Chem. Soc.* **79**, 3222 (1957).
[13] For example see: D. N. Kirk, D. K. Patel and V. Petrow, *J. Chem. Soc.* 1046 (1957); and V. Schwarz and K. Syhora, *Collection Czech. Chem. Commun.* **28**, 637 (1963).
[14] R. B. Woodward, F. Sondheimer and Y. Mazur, *J. Amer. Chem. Soc.* **80**, 6693 (1958).
[15] H. Budzikiewicz, J. M. Wilson and C. Djerassi, *Monatsh. Chem.* **93**, 1033 (1962).

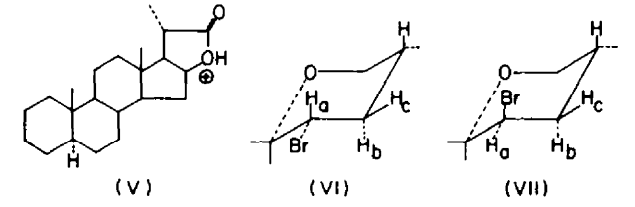
study have been reported by one of us.[16] We now describe NMR data pertinent to the bromination problem which allows unequivocal structures to be presented for the bromotigogenin isomers. The NMR data also indicates that NMR spectroscopy is a very useful technique for problems of this type.



(I) (II)



(III) a, R=H ; b, R=OAc (IV) a, R=H ; b, R=OAc



(V) (VI) (VII)

The recent NMR study[16] of steroidal sapogenins emphasized that two regions of the spectra would be particularly important for solution of the present problem: (a), the high field region involving the C-methyl signals and (b), the 200–300 c/s region, encompassing signals for protons at C-16, C-23 and C-26. For the sake of clarity, each region is discussed separately in the sequel.

The C-methyl region

Figure 2 shows the high field region of compounds IIIa–IVb and several features are immediately apparent. First, the spectra reveal a normal C-methyl region typical of the steroidal sapogenins. Using previous results[16] analysis of this region is straightforward. The methyl group at C-25 occurs as a doublet at highest field and normally only one signal of this doublet is clearly evident (44 c/s and 45 c/s in IIIa and IIIb, Fig. 2). The angular methyl (C-18 and 19) signals usually appear as two very sharp peaks and in the present cases are seen at 47 and 51 c/s, and 50 and 53 c/s respectively. Usually only one signal of the doublet predicted for the C-21 methyl protons is clearly evident at slightly lower field, while the other signal is hidden under

[16] J. P. Kutney, *Steroids* **2**, 225 (1963).

signals presented by the angular methyl protons. In this case, the response at 57 c/s (in IIIa) and at 58 c/s (in IIIb) correspond to part of the C-21 doublet. It is also important to emphasize that the total area under the above signals corresponds to twelve protons.

Consideration of the C-methyl region for compounds IVa and IVb reveals that in both instances, the C-21 methyl proton signals appear further downfield. The

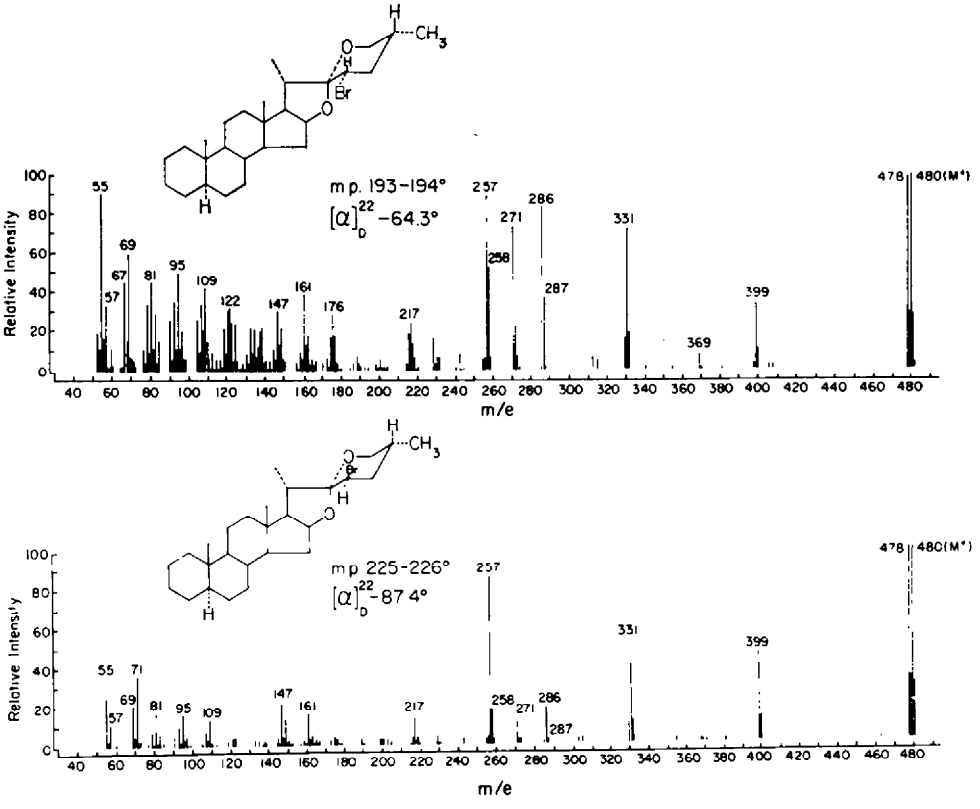


FIG. 1

expected doublet (J = 7 c/s) is now well separated from the other methyl proton signals (66 and 73 c/s in IVa and 67 and 74 c/s in IVb). Further confirmation of this shift is evident from a careful integration of areas under the high field (below 50 c/s) signals which correspond to *nine* protons. Finally, the NMR spectrum of IVb obtained at 100 mc/s (discussed below) confirmed that the separation of 7 c/s mentioned above, was indeed indicative of a spin-spin interaction since this value remained *unchanged* in the 100 mc/s spectrum.

In view of the above evidence, it was clear that in compounds IIIa and IIIb, the bromine atom must be situated in the side-chain in such a manner as to have essentially no effect on the chemical shifts of protons in the methyl groups. However in the case of IVa and IVb, the bromine atom must be in reasonable proximity to the C-21 methyl group in order to cause the observed downfield shift. Molecular models indicate that if we assume C-23 as the site of bromination, a C-23 equatorial bromine

atom would have little effect whereas a C-23 axial bromine substituent could cause a downfield shift of the C-21 signals.

The above NMR data also completely eliminates C-20 as a possible position for the bromine atom. A C-20 bromo derivative would display a single sharp signal for the C-21 methyl group at lower field: a situation inconsistent with any of the spectra shown in Fig. 2. Similarly, C-25 bromo derivatives are also eliminated from consideration. In all spectra reproduced in Fig. 2, the highest field signal (occurring at 44–46 c/s) was assigned to part of the doublet expected for the C-27 equatorial methyl protons.

The 200–300 c/s *region*

As pointed out in the previous NMR investigation,[16] an analysis of the 200–300 c/s region particularly with regard to splitting patterns corresponding to C-26 protons can provide direct information about the stereochemistry at C-25. Consideration of the spectra shown in Figs 3 and 4 shows that the normal situation generally observed in the 25D series prevails: a broad set of signals approximating a doublet is observed. The presence of this pattern also excludes a C-25 bromo substituent. However, of greater importance to the present problem was analysis of the signals occurring at lower field than those due to the C-26 protons.

In order to assign signals in the downfield region, we first analyzed the NMR spectrum of desoxytigogenin (not shown) and, as expected, the lone proton at C-16 was found to occur as a broad set of lines in the 250–275 c/s region. The assignment was substantiated by viewing the low field region displayed by tigogenin acetate. In this case, a much broader set of signals (corresponding in area to two protons) was noted in the region, 250–300 c/s, as expected for the C-16 and C-3 protons. From these results it was obvious that the broad set of lines arising from the C-3 proton was occurring at slightly lower field than those from the C-16 proton, but the small difference in chemical shift merely caused a broadening of the multiplet in this region.

Turning again to the spectra in Figs 3 and 4, one observes a striking difference in the low field region of IIIa and IIIb as compared to IVa and IVb. First we considered this region (235–270 c/s) in the spectrum of bromosapogenin IIIa. The spectrum shows a new set of signals for a single proton, apart from the signals already mentioned for the C-16 proton in desoxytigogenin, and these must represent a lone proton bonded to the carbon bearing the bromine substituent. Furthermore, the signals encompassed by the region 235–250 c/s, as shown in Fig. 3, approximate a quartet and resemble the low field spectrum of bromotigogenin IIIb. In fact, the splitting pattern at 235–270 c/s is nearly identical to that shown by bromodesoxytigogenin IIIa and the additional broad multiplet at slightly lower field (270–300 c/s) is due to the C-3 proton. By contrast, Fig. 4 which illustrates the low field region of the remaining two bromo isomers (in the desoxytigogenin and tigogenin series) reveals a strikingly different splitting pattern. In the spectrum of isomer IVa two rather distinct regions are evident. The C-16 proton signals occur again as a broad set at 260–285 c/s and a masked triplet due to one proton is centered at 247 c/s. Evidently the triplet at 247 c/s is due to a proton on the bromine substituted carbon atom. The spectrum of IVb in the same region is identical with that of IVa and the broad multiplet for the C-3 proton appears on the low field side (near 300 c/s) of the C-16 proton signals.

The above data completely eliminates C-20 and C-25 as possible sites for the

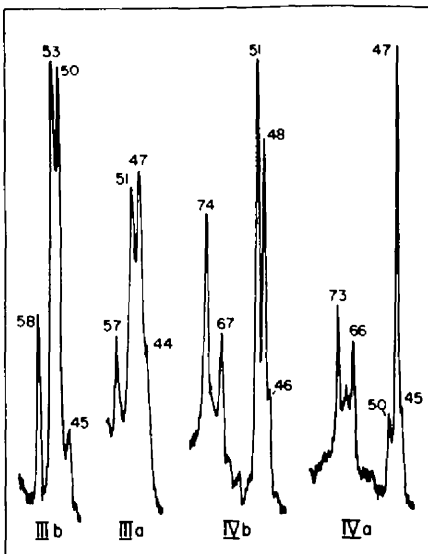


Figure 2. High field region of nuclear magnetic resonance spectra at 60 Mc/s.

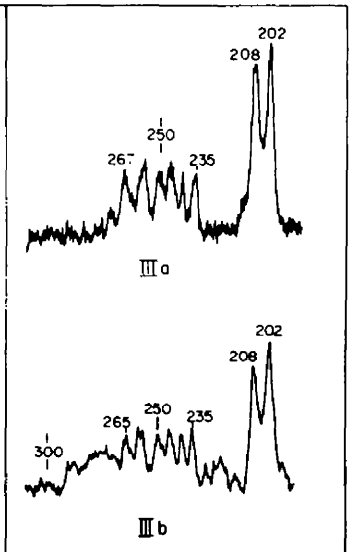


Figure 3. Low field region of nuclear magnetic resonance spectra at 60 Mc/s.

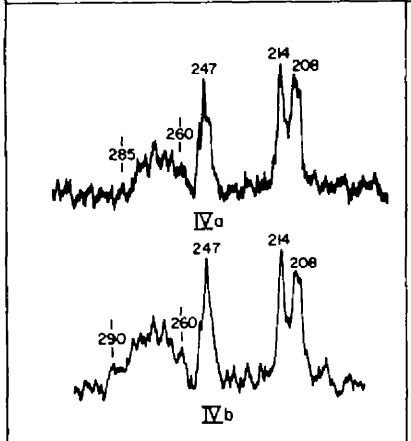


Figure 4 Low field region of nuclear magnetic resonance spectra at 60 Mc/s

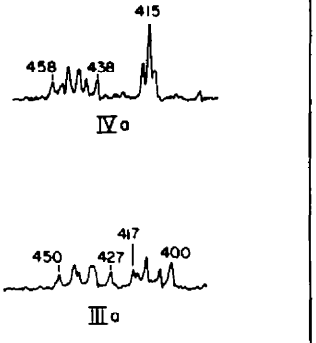


Figure 5. Low field region of nuclear magnetic resonance spectra at 100 Mc/s.

FIGS. 2, 3, 4 and 5

bromine substituent and leads to the conclusion that introduction of bromine into the spiroketal system must involve a methylene carbon at C-23, C-24 or C-26. However, the C-26 proton responses at 200–215 c/s (*vida supra*) exclude this position. Since there is no chemical reason to consider bromination at C-24, the *bromine substituent must be at* C-23. The only remaining problem was to establish which of the related pairs of isomers possess an equatorial and which an axial bromine configuration at C-23.

With an equatorial bromine atom, the C-23 axial proton would enter into axial-axial and axial-equatorial spin-spin interactions with two protons on the adjacent

C-24 carbon atom. The coupling constant for an axial-axial interaction, J_{ab}, is larger than J_{ac} (see VI) and the former is normally assigned a value of about 6–12 c/s whereas the latter is given a value of about 3–5 c/s.[17,18] Thus, the splitting pattern expected for a C-23 axial proton would be a set of four signals of approximately equal intensity and each separated from an adjacent signal by the appropriate number of cycles per second. The patterns actually observed in the spectra of IIIa and IIIb (235–250 c/s) closely approximate the theoretical case.

A C-23 equatorial proton would produce interactions, with the C-24 protons, of the equatorial-equatorial and equatorial-axial type. Since J_{ab} and J_{ac} (see VII) are approximately equal and are normally given a value of about 3–5 c/s[17,18] the splitting pattern might appear as a triplet if the right conditions prevail.[19] Indeed, the patterns observed in the spectra of IVa and IVb are approximately triplets centered at 247 c/s with a half-height width of 7 c/s.

NMR spectra at 100 *mc/s*

In order to obtain more clearly resolved spectra particularly in the low field region, the NMR spectra of desoxytigogenin and its two isomeric bromo derivatives were obtained using a 100 mc/s instrument. As shown in Fig. 5, the signals at low field are well separated and serve to confirm the assignments already made using the 60 mc/s data. Desoxytigogenin (not shown) displayed a broad set of lines in the region, 425–450 c/s, corresponding to the lone proton at C-16. The C-23 equatorial bromo isomer (IIIa) produced in addition to the C-16 proton signals, a new broad multiplet due to the C-23 axial proton at 400–417 c/s. Integrated areas under these signals were in agreement with lone protons at these positions. A more striking illustration of the advantage of NMR spectra at 100 mc/s is shown by the low field region exhibited by isomer IVa. NMR response of the C-23 proton appears as a clearly defined triplet centered at 415 c/s as expected for the isomer with an axial bromine atom at C-23.

The above NMR data provides conclusive evidence for the structural and stereochemical assignments of all four isomers studied in the bromotigogenin series. In summary, the isomer melting at 193–194° in the desoxytigogenin sequence and the one melting at 206–208° in the tigogenin acetate series have been assigned structures IIIa and IIIb respectively. The other pair of isomers have been assigned structures IVa and IVb.

EXPERIMENTAL

Pet. ether refers to a petroleum ether fraction boiling at 40–50°. M.ps were observed using a Kofler m.p. apparatus. Elemental microanalyses were performed in the laboratories of Dr. A. Bernhardt, Max Planck Institute, Mulheim, Germany. Mass spectral results[20] were obtained using an Atlas-Mat Model CH4 mass spectrometer equipped with a direct inlet system. Ionizing energy of the mass spectrometer was maintained at 70 ev and the ionizing current at 50 μa.

[17] L. M. Jackman, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry* pp. 84–86. Pergamon Press (1959).

[18] A. C. Huitric, J. B. Carr, W. F. Trager and B. J. Nist, *Tetrahedron* **19**, 2145 (1963).

[19] K. B. Wiberg and B. J. Nist, *Interpretation of NMR Spectra* p. 21. W. A. Benjamin, Inc. (1962).

[20] We wish to thank Professor C. Djerassi, Chemistry Department, Stanford University for running the mass spectra.

The NMR spectra at 60 mc/s and 100 mc/s[21] were recorded employing, respectively, Varian Associates Models A-60 and HR-100 NMR spectrometers. The spectra were taken in deuteriochloroform solutions with tetramethylsilane used as an internal standard. Values are given in cycles/sec (c/s) with respect to the TMS signal set at 0 c/s. IR spectral data was provided by Dr. R. A. Hill (University of Maine) and optical rotation (chloroform solution) measurements by Drs. Weiler and Strauss, Oxford, England.

Bromination of 5α,25*R-spirostan* (I).[22] A solution of Br_2 (2·9 g) in glacial acetic acid (36 ml) was added (over 30 min) to a solution (maintained at 60°) composed of desoxytigogenin (6·0 g),[7] 2 drops of 4N HBr in glacial acetic acid, and glacial acetic acid (800 ml). A deep blue colour[23] formed at once and intensified upon further addition of Br_2. The mixture of liquid and crystals (separated during bromination) was allowed to remain at room temp for ca. 24 hr. After filtration, the crystalline product was separated into two principal components (3·7 g, m.p. 186–196° and 1·0 g, m.p. 211–222°) by fractional recrystallization from chloroform (Norit-A)-methanol. Repeated recrystallization of the lower melting mixture from chloroform–pet. ether eventually gave a pure specimen of 23S-*bromo*-5α,25R-*spirostan* melting at 193–194°; $[\alpha]_D^{22}$ −64·3° (*c*, 1·24), ν_{max}^{KBr} 1008, 950, 918, 865 and 730 cm^{-1}. (Found: C, 67·42; H, 8·75; Br, 16·91; mol. wt, 480. $C_{27}H_{43}BrO_2$ requires: C, 67·60; H, 9·00; Br, 16·70%; mol. wt 480.) Similar treatment of the higher melting fraction yielded an analytical sample of IVa melting at 215–217° to 225–226° (depending on rate of heating); $[\alpha]_D^{22}$ −87·4° (*c*, 1·36), ν_{max}^{KBr} 1015, 972, 943, 905 and 880 cm^{-1}. (Found: C, 67·36; H, 8·84; Br, 17·01%; mol. wt 480.)

[21] We wish to thank Professor W. A. Ayer, Chemistry Department, University of Alberta, for running the NMR spectra at 100 mc/s.

[22] We wish to acknowledge the contributions of Dr. T. R. Kasturi during an early phase of this study.

[23] Cf., G. P. Mueller, L. L. Norton, R. E. Stobaugh, L. Tsai and R. S. Winniford, *J. Amer. Chem. Soc.* **75**, 4892 (1953).