532. *Studies in the Sterol Group. Part LII.* Infra-red Absorption of Nuclear Tri- and Tetra-substituted Ethylenic Centres.*

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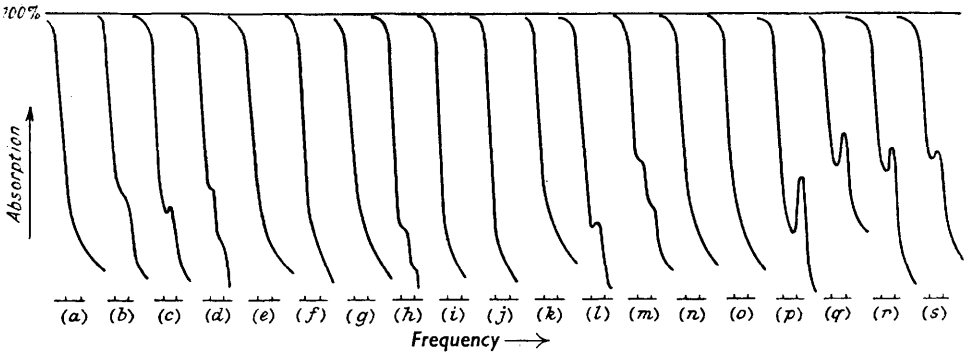
The infra-red spectra of nineteen singly unsaturated steroids, providing examples of all the possible tri- and tetra-substituted ethylenic positions in the nucleus except Δ^{16}, have been recorded in the three characteristic olefinic-absorption regions of 3·3, 6, and 10—15 μ. under comparable conditions of path-length and concentration. The tetra-substituted ethylenes (hydrocarbons, alcohols, and ketones) are transparent at these wave-lengths, whereas the tri-substituted derivatives display more or less characteristic absorption bands of weak or moderate intensity in all three spectral regions. The occurrence of a tri-substituted ethylenic centre can therefore in general be recognized, and its location further specified, by means of significant frequency and intensity differentiation at 3·3 and at 6 μ. The electronic nature of steric strain accompanying the presence of unsaturation in rings C or D of the steroid nucleus, and its spectroscopic consequences, are discussed.

The location of unconjugated ethylenic double bonds in the nucleus of synthetic or naturally occurring compounds of the sterol group presents a diagnostic problem which clearly demands a thorough exploration of the possibilities inherent in spectroscopic techniques. So far, little systematic work has been done in this field. Ultra-violet studies have been restricted to the

Fig. 1.

Transmitted spectral energy records at 3·3 μ.



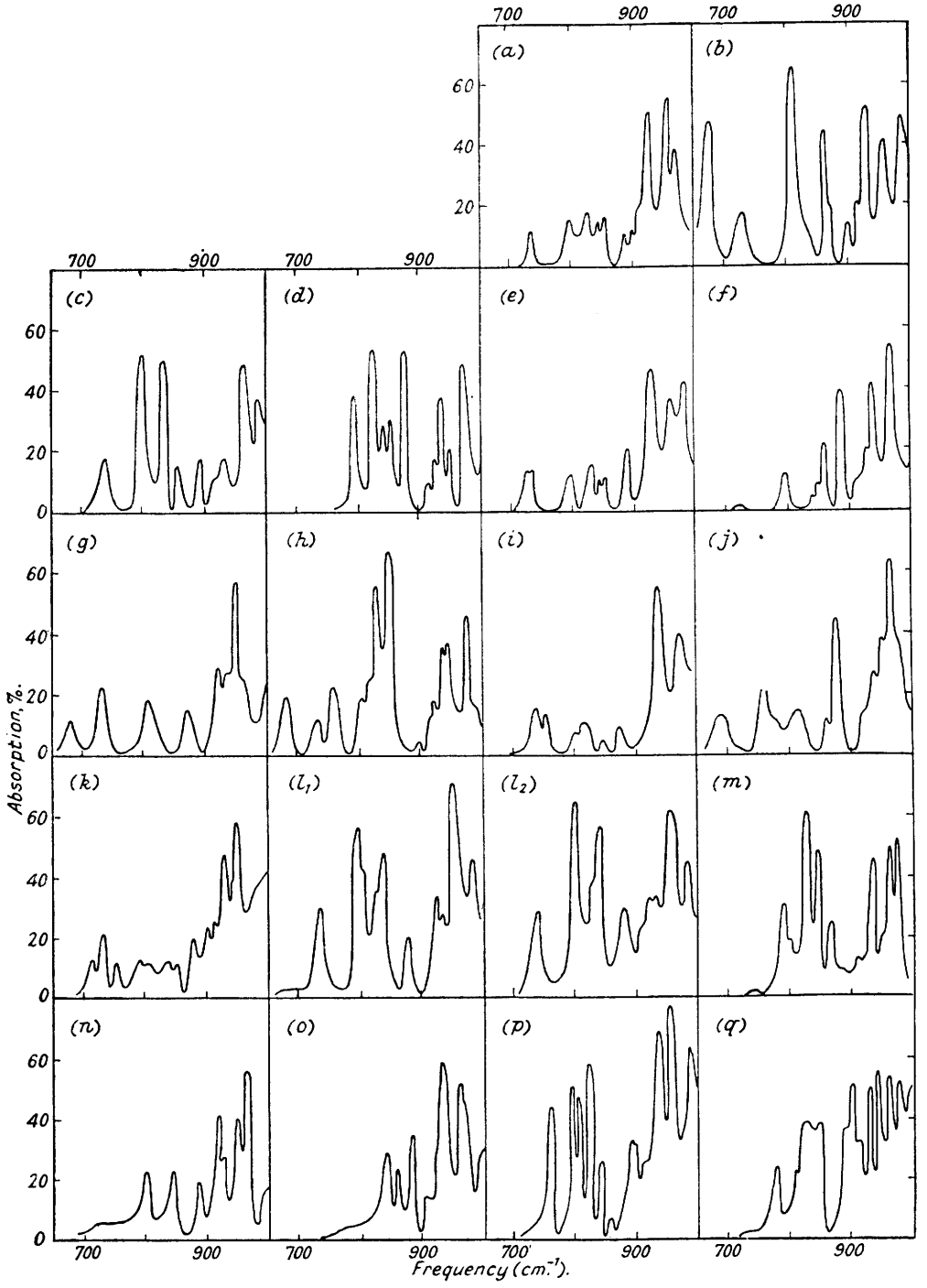
Calibration marks refer to 3030 *and* 3040 *cm.*$^{-1}$. *Solutions in carbon tetrachloride: effective path-length of solute* ~0·035 *mm. For sample references, a—s, see table.*

instrumentally more accessible absorption region of conjugated double bonds; quartz ultra-violet measurements on unconjugated ethylenic centres, undertaken here concurrently with the present work, will be reported in a separate communication. In the infra-red region, well-tried diagnostic methods were available only for non-cyclic compounds when this project was initiated, but a notable contribution to the characterization of isolated double bonds in steroid molecules was more recently published by Jones, Humphries, Packard, and Dobriner (*J. Amer. Chem. Soc.*, 1950, **72**, 86). These authors investigated the 6-μ. region of isolated Δ^{2}-, Δ^{5}-, Δ^{7}-, $\Delta^{8(14)}$-, $\Delta^{9(11)}$-, Δ^{11}-, Δ^{14}-, and Δ^{16}-ethylenic centres in a number of steroid molecules (mostly containing carbon–oxygen double bonds). They also reported some preliminary data on the 3·3-μ. region (cf. Jones, Williams, Whalen, and Dobriner, *ibid.*, 1948, **70**, 2024) and drew attention to the diagnostic possibilities of the 10—12-μ. range.

* Part LI, *J.*, 1951, 1190.

FIG. 2.

Absorption spectra at 10—15 μ.

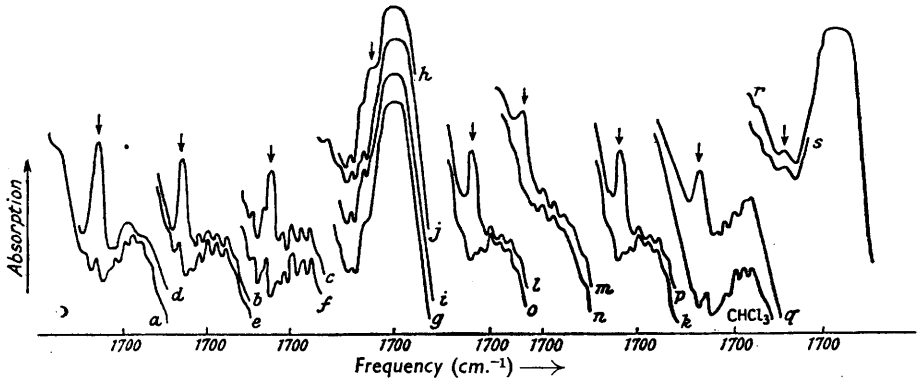


a—l$_1$, *p*, *q in carbon disulphide solution :* *l*$_2$, *m—o in Nujol suspension. Effective path-length of steroid sample* ~0·045 *mm. For sample references, a—q, see table.*

Characteristic infra-red absorption of olefinic groupings arises from the stretching vibrations of the double bonds and of any adjacent C–H bonds (near 6 and 3·3 μ. respectively), and also from the out-of-plane bending vibrations of these olefinic C–H bonds (at 10—15 μ.). As is well known, all three modes of vibration occur approximately independently of the rest of the structure in aliphatic hydrocarbon molecules, and this appears to be true also when the double bonds form part of a strainless ring. Thus, *cyclo*hexene displays the characteristic bands of *cis*-disubstituted double bonds in straight-chain olefins, and 1-methyl- and 1-methyl-4-*iso*propenyl-*cyclo*hexene give rise to typical trialkylethylene frequencies in the three characteristic spectral regions (Fox and Martin, *Proc. Roy. Soc.*, 1940, *A*, **175**, 208; American Petroleum Institute, "Catalog of Infra-Red Spectrograms;" and our own observations). The most easily characterized, because the most intensely absorbing, are the terminal methylene and the *trans*-disubstituted ethylenic types, but these do not occur in the steroid nucleus. Our observations on *cis*-disubstituted cyclic double bonds such as occur in Δ^{2}- and Δ^{11}-sterols will be reported in a subsequent paper. As regards the tri- and tetra-substituted ethylenic centres in the steroid nucleus, Jones, Humphries, Packard, and Dobriner (*loc. cit.*) have already drawn attention to the weakness or absence of characteristic infra-red frequencies at 3·3 and 6 μ. in many cases, and also to the difficulty of their certain detection at 6 μ. in the presence of

FIG. 3.

Transmitted spectral energy records at 6 μ.



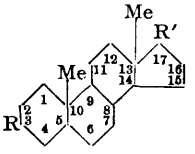
Solutions in chloroform: effective path-length of solute ~0·2 mm. For sample references, a—s, see table. Arrows indicate characteristic bands.

strongly absorbing carbon–oxygen double bonds or of water-vapour in the spectrometer. It therefore still seemed important independently to check, as well as to extend, their findings, preferably in the absence of interfering absorptions, and to study also the 10—15-μ. diagnostic region in order to be able to make full use of all available spectroscopic information in doubtful cases.

Infra-red Spectra–Structure Correlation Rules for Tri- and Tetra-substituted Ethylenes, and their Application to the Steroid Group.—Tetra-substituted ethylenes do not contain C–H bonds adjacent to the double bond and therefore cannot give rise to characteristic absorption frequencies in either the 3·3-μ. (3000—3100-cm.$^{-1}$) or the 10—15-μ. (650—1000-cm.$^{-1}$) spectral regions. In the double-bond stretching region at 6-μ. (1620—1700-cm.$^{-1}$), infra-red absorption of such olefins is excessively weak or absent owing to the high degree of symmetry around the tetra-substituted linkage. Thus, liquid tetramethylethylene shows only a very small band at 1684 cm.$^{-1}$ (A.P.I., *loc. cit.*), and no maximum could be observed for cholest-8(14)-enol (I; R = OH, C_8–C_{14} ethylenic) by Jones *et al.* (*loc. cit.*, 1950). The less symmetrically trisubstituted ethylenic compounds are generally characterized by a band of moderate to weak intensity at 1660—1675 cm.$^{-1}$ such as has been found in a considerable number of simple straight-chain olefins (A.P.I., *loc. cit.*), in acyclic terpenes and polyisoprenes (Barnard, Bateman, Harding, Koch, Sheppard, and Sutherland, *J.*, 1950, 915; Saunders and Smith, *J. Appl. Physics*, 1949, **20**, 953), in 1-methyl- and 1-methyl-4-*iso*propenyl-*cyclo*hexene (A.P.I., *loc. cit.*, and our own observations), and by Jones *et al.* (*loc. cit.*, 1950) also in cholesterol (I; R = OH, C_5–C_6 ethylenic) and cholest-7-enol (I; R = OH, C_7–C_8 ethylenic). The single olefinic C–H group of trisubstituted ethylenes gives rise to moderate or weak characteristic absorption in

the 3·3-μ. C–H stretching region near 3050 cm.$^{-1}$ (Fox and Martin, *loc. cit.*; Saunders and Smith, *loc. cit.*; cf. also Jones *et al.*, *loc. cit.*, 1948), and also to a well-known moderate or strong and rather variable out-of-plane bending frequency at 790—850 cm.$^{-1}$ (12 μ.) which apparently lies at 796—798 cm.$^{-1}$ in 1-methyl- and 1-methyl-4-*iso*propenyl-*cyclo*hexene (A.P.I., *loc. cit.*, and our own observations), at 790 cm.$^{-1}$ in α-pinene (II) (A.P.I., *loc. cit.*), and at 803 or 844 cm.$^{-1}$ in cholesterol (I; R = OH, C_5–C_6 ethylenic) (Baird, O'Bryan, Ogden, and Lee, *J. Opt. Soc. America*, 1947, **37**, 754; Hainer, King, and McMahon, *Physical Rev.*, 1949, **75**, 1320).

(I.)



(II.)

Chemical methods can usually be applied to establish the presence or otherwise of a double bond in the sterol nucleus without much difficulty. Ideally, then, we might expect the tetra-substituted ethylenic groups to be uniquely characterized by relative or complete transparency in all three diagnostic frequency regions and the $\Delta^{8(9)}$- and $\Delta^{8(14)}$-compounds to be indistinguishable in this respect; we might expect the trisubstituted ethylenic derivatives to give bands near 3050 cm.$^{-1}$ and to be uniquely characterized by absorption at 1660—1675 and 790—850 cm.$^{-1}$ with possibly minor characteristic variations amongst the several members of this group (Δ^{4}-, Δ^{5}-, Δ^{7}-, $\Delta^{9(11)}$-, Δ^{14}-, or Δ^{16}-ethylenic).

The above spectra–structure correlation rules for olefinic groupings appear to have been strictly established for hydrocarbons only, and it therefore seemed desirable to examine a number of pure samples of steroid hydrocarbons (I; R = H, R′ = C_nH_{2n+1}) as well as the usual functional derivatives, so that any disturbing influence of functional groups on the olefinic spectrum might be recognized and, if need be, eliminated. Exploratory infra-red spectroscopic work in the sterol group is also greatly facilitated by the use of purely hydrocarbon compounds on account of their ready solubility in spectroscopically suitable solvents. It is preferable to work with solutions rather than solid films or suspensions in order that relative absorption intensities may be more conveniently estimated and compared between different substances, crystal structure effects eliminated, and sufficiently long effective path-lengths employed without undue loss of radiation by scattering.

Preparative Work.—Steroid hydrocarbons containing a double bond at positions 4, 5, 7, 8(9), and 8(14) have been prepared. Since hydroxyl groups appear to exert no detectable influence on the infra-red absorption of double bonds separated by at least 3 carbon atoms (cf. ergost-7-ene and 3β-hydroxyergost-7-ene), 3β-hydroxyergost-14-ene and 24-hydroxychol-9(11)-ene were prepared as examples of their respective double-bond types.

The route employed for preparing the parent hydrocarbons containing double bonds in the 7-, 8(9)-, and 8(14)-positions was to oxidise the readily accessible 3β-sterols to the corresponding ketones, which were reduced to hydrocarbons by the Kishner–Wolff method.

The chosen starting material for ergost-7- and 8(14)-ene was α-dihydroergosterol, this compound being obtained as a by-product from the irradiation of ergosterol which normally contains a few units % of the α-dihydro-compound as an impurity unaffected by the irradiation process. Hydrogenation of the side-chain double bond in α-dihydroergosterol to give 3-hydroxyergost-7-ene was effected in excellent yield in the presence of a Raney nickel catalyst at normal temperature and pressure, this method being found superior in yield and reproducibility to those employing platinum oxide catalysts. Use of a Raney nickel catalyst at higher temperatures and pressures resulted in isomerization of the nuclear double bond to the 8(14)-position, good yields of 3-hydroxyergost-8(14)-ene being obtained.

Oxidation of the above sterols to the corresponding ketones was carried out by chromic acid in acetone (cf. Bowden, Heilbron, Jones, and Weedon, *J.*, 1946, 39). No attack on the double bonds was then detected, the ketones being formed in good yield, accompanied by some unchanged sterol. The Kishner–Wolff reductions proceeded smoothly. The properties of ergost-8(14)-ene agreed with those given for this compound by Heilbron, Spring, and Webster (*J.*, 1932, 1705), who prepared it by sodium–alcohol reduction of the 3-chloro-compound.

A hydrocarbon containing an 8(9)-double bond [cholest-8(9)-ene] was conveniently prepared from zymosterol, which was isolated from the non-saponifiable part of yeast fat *via* its 24 : 25-dibromide (cf. Heath-Brown, Heilbron, and Jones, *J.*, 1940, 1182). It was possible to increase

considerably the scale of the dibromide formation by suitable cooling during the addition of bromine. Hydrogenation of zymosterol under mild conditions gave 3β-hydroxycholest-8(9)-ene, oxidized in this instance by the Oppenauer method to the corresponding ketone. Kishner–Wolff reduction then gave cholest-8(9)-ene.

Experimental.

M. p.s were determined on a Kofler block and are corrected. Optical rotations were determined in chloroform solution in a semi-micro-tube of length 1 dm. at room temperature (18—25°) unless stated otherwise. Samples for analysis and determination of physical constants were dried *in vacuo* at 115° or at 20° below the m. p. The alumina (P. Spence, Grade O) used for chromatography had an activity between 2 and 3 on the Brockmann–Schodder scale (*Ber.*, 1941, **74**, 75).

Cholest-4-ene.—This was prepared by Raney nickel desulphurization of the dibenzyl mercaptal of 3-ketocholest-4-ene (method of Hauptmann, *J. Amer. Chem. Soc.*, 1947, **69**, 562). By use of the more active W4 grade of Raney nickel (Adkins and Pavlic, *ibid.*, 1946, **68**, 1471) desulphurization could be effected at 20°. The mercaptal (1 g.) in dioxan (50 c.c.) was stirred for 7½ hours at 20° with W4 Raney nickel (5 g.). The product after recrystallization had m. p. 79·5—81°, $[\alpha]_D$ +68° (*c*, 1·28) (lit., m. p. 78—79°, $[\alpha]_D$ +65°). Dr. D. H. R. Barton kindly informed us that such cholest-4-ene contained an impurity (probably cholest-3-ene), and therefore for further purification the above product was converted into its dibromide (Mauthner, *Monatsh.*, 1907, **28**, 1113), which after recrystallization from benzene–ethanol formed needles, m. p. 117—119° (Mauthner gives m. p. 116—117°). A solution of the dibromide (275 mg.) and sodium iodide (500 mg.) in acetone (10 c.c.) was heated under reflux for 30 minutes. Cooling and dilution with water gave a crystalline product, which after recrystallization from ethanol gave pure cholest-4-ene as needles, m. p. 82—83·5°, $[\alpha]_D$ +76° (*c*, 0·7).

Cholest-5-ene.—This was prepared by the method of Mauthner and Suida (*ibid.*, 1894, **15**, 85). The product obtained was purified by chromatography to give cholest-5-ene of m. p. 92·5—94°, $[\alpha]_D$ −56° (*c*, 1·01) (lit., m. p. 88—90°, $[\alpha]_D$ −56°).

3β-Hydroxycholesta-8(9) : 24-diene (Zymosterol).—This sterol was obtained *via* its dibromide from the non-saponifiable material from yeast fat after the bulk of the ergosterol had been removed by crystallization. Subsequent purification of the crude dibromide was facilitated by partial purification of this crude gummy mixture (1 kg.) by trituration with (*a*) ethanol (1·1 l.) and (*b*) methanol (750 c.c.). This gave a light brown solid (720 g.) containing 20—25% of ergosterol, estimated spectroscopically. It was found possible to increase considerably the scale of the next stage by suitable cooling. The purified sterol mixture (75 g.) in dry ($CaCl_2$) ether (1·5 l.) was cooled to −10°; a 10% solution of bromine in acetic acid (450 c.c.) was then added with vigorous shaking during 1 minute, the internal temperature being kept at −5° to −10° by external cooling with solid carbon dioxide. The mixture was then set aside in an ice-bath until the supernatant liquid became dark (5—10 minutes). The product was rapidly filtered off and washed with ethanol (30 c.c.) and then ether (100 c.c.). Recrystallization from ethanol–chloroform (3 : 1) and ethyl acetate gave zymosterol dibromide (7—8 g.) as needles, m. p. 157—158°, $[\alpha]_D$ +7° (*c*, 1·89). Heath-Brown, Heilbron, and Jones give m. p. 157°, $[\alpha]_D$ +7·4° (*c*, 3·3).

Debromination of the dibromide with zinc dust and absolute ethanol gave zymosterol, m. p. 109°, $[\alpha]_D$ +49° (*c*, 3·10); Heath-Brown *et al.* give m. p. 107—109°, $[\alpha]_D$ +49° (*c*, 1·4).

3β-Hydroxycholest-8(9)-ene.—A solution of zymosterol (3 g.) in benzene (150 c.c.) was shaken with hydrogen at atmospheric pressure in the presence of Raney nickel (3 g.) until no more was absorbed. Evaporation of the solvent, after removal of the catalyst by filtration through a sintered-glass funnel, gave a solid, which after recrystallization from methanol afforded the sterol (2·7 g.) as flat plates, m. p. 128°, $[\alpha]_D$ +49° (*c*, 2·79). Wieland, Rath, and Benend (*Annalen*, 1941, **548**, 19) give m. p. 129°, $[\alpha]_D$ +50°.

3-Ketocholest-8(9)-ene.—A solution of the foregoing sterol (2·2 g.) in dry acetone (20 c.c.) was added to aluminium *tert.*-butoxide (2·5 g.) in dry benzene (50 c.c.), the mixture then being heated under reflux for 8 hours. After the addition of water, the steroid was isolated with ether. Traces of mesityl oxide were removed by the addition of xylene (7·5 c.c.), followed by evaporation *in vacuo*. The product was chromatographed on alumina (100 g.). Development with benzene gave a product which after recrystallization from methanol yielded *3-ketocholest-8(9)-ene* (1·35 g.) as flat needles, m. p. 119·5—120·5°, $[\alpha]_D$ + 69° (*c*, 2·28) (Found: C, 84·0; H, 11·5. $C_{27}H_{44}O$ requires C, 84·3; H, 11·5%). Elution of the chromatogram with ether–methanol gave unchanged sterol (0·22 g.), m. p. 127°.

Cholest-8(9)-ene.—A mixture of 3-ketocholest-8(9)-ene (750 mg.), hydrazine hydrate (7 c.c. of 60% w/v), and sodium (1·25 g.) dissolved in ethanol (25 c.c.) was heated (autoclave) for 8 hours at 210—220°. The steroid was isolated with light petroleum (b. p. 40—50°), the solution being passed through a column of alumina (75 g.), which was developed further with light petroleum (b. p. 40—50°). Evaporation of the eluant gave a solid, which after recrystallization from methanol afforded *cholest-8(9)-ene* (420 mg.), m. p. 92—93·5°, $[\alpha]_D$ +56° (*c*, 1·09) (Found: C, 87·6; H, 12·55. $C_{27}H_{46}$ requires C, 87·5; H, 12·5%).

3β-Hydroxyergosta-7 : 22-diene (α-Dihydroergosterol).—Crude α-dihydroergosterol, obtained as a residue from the irradiation of ergosterol, was purified *via* its acetate. Recrystallization of the acetate from benzene–acetone gave platelets, m. p. 180—181°, $[\alpha]_D$ −23° (*c*, 2·0). Barton and Cox (*J.*, 1948, 1354) give m. p. 181°, $[\alpha]_D$ −20° (*c*, 1·78). Alkaline hydrolysis of the acetate gave α-dihydroergosterol (microscopic needles from chloroform–methanol), m. p. 176—178°, $[\alpha]_D$ −23° (*c*, 2·1). Barton and Cox give m. p. 176°, $[\alpha]_D$ −19° (*c*, 1·8).

3β-Hydroxyergost-8(14)-ene (α-Ergostenol).—The acetate of this sterol was prepared by hydrogenation of 3β-acetoxyergosta-7 : 22-diene, either in acetic acid–ether with a platinum oxide catalyst at 100°/100

atm., or by the following new method. The diene-acetate (2 g.) in ethyl acetate (300 c.c.) was stirred in an autoclave with hydrogen at 100°/100 atm. in the presence of Raney nickel (3·5 g.) for 12 hours. The catalyst was removed by filtration through a pad of alumina. Recrystallization from ethyl acetate–methanol yielded the pure acetate (1·8 g.) as plates, m. p. 110—112°, $[\alpha]_D$ −1° (*c*, 2·15). Barton and Cox (*J.*, 1948, 783) give m. p. 109°, $[\alpha]_D$ +1° (*c*, 2·13).

Alkaline hydrolysis of the acetate gave 3β-hydroxyergost-8(14)-ene, which after recrystallization from chloroform–methanol formed small needles, m. p. 132·5—134°, $[\alpha]_D$ +10° (*c*, 1·95). Barton and Cox (*loc. cit.*) give m. p. 132°, $[\alpha]_D$ +11°.

3-*Ketoergost*-8(14)-*ene*.—The chromic acid solution (*ca.* 8N.) used as the oxidizing agent was prepared by dissolving pure chromium trioxide (66·7 g.) in water, adding concentrated sulphuric acid (53·3 c.c.), and diluting the mixture to 250 c.c. with water. A solution of 3β-hydroxyergost-8(14)-ene (5 g.) in AnalaR acetone (500 c.c.) was stirred vigorously at 30—35°. Chromic acid solution (10 c.c.) was added during 3 minutes, and after a further minute's stirring the excess of oxidant was destroyed by addition of a solution of sulphur dioxide in acetone. Saturated aqueous potassium carbonate was added, together with ether, and the acetone–ether layer separated. Drying and evaporation of this layer gave a product (4·2 g.) which was chromatographed on alumina (100 g.). Elution with benzene gave the ketone, which after recrystallization from ethanol–methanol formed platelets (3 g.), m. p. 128—130°, $[\alpha]_D$ +29° (*c*, 2·4). Barton and Cox (*loc. cit.*) record m. p. 129—130°, $[\alpha]_D$ +30° (*c*, 1·91).

Ergost-8(14)-*ene*.—The foregoing ketone (1 g.) was heated with hydrazine hydrate (7 c.c. of 60% w/v) and a solution of sodium (1·25 g.) in ethanol (25 c.c.) in an autoclave at 190—215° for 5 hours. The steroid was isolated with pentane and chromatographed on alumina (50 g.). Elution with pentane gave ergost-8(14)-ene (0·86 g.) which after recrystallization from ethyl acetate–methanol formed microscopic prisms, m. p. 79·5—81°, $[\alpha]_D$ +13° (*c*, 3·0). Heilbron, Spring, and Webster (*loc. cit.*) give m. p. 77—78°, $[\alpha]_D$ +11° (*c*, 1·0).

3β-*Hydroxyergost*-7-*ene* (γ-*Ergostenol*).—Difficulty was encountered in the preparation of the acetate of this sterol by hydrogenation of 3β-acetoxyergosta-7 : 22-diene in ethyl acetate solution with a platinum oxide catalyst owing to incomplete hydrogenation. However, use of a Raney nickel catalyst gave high and reproducible yields. A solution of the acetoxy-diene (4·4 g.) in ethyl acetate (350 c.c.) (kept at 30° initially in order to keep the solid in solution) was shaken with Raney nickel (1·5 g.) in hydrogen at atmospheric pressure until no more hydrogen was taken up (20 hours). The product was recrystallized from chloroform–methanol and ethyl acetate–methanol, to give 3β-acetoxyergost-7-ene (4·0 g.) as plates, m. p. 158—160°, $[\alpha]_D$ −3° (*c*, 4·1). Barton and Cox (*loc. cit.*) give m. p. 157—159°, $[\alpha]_D$ −4° (*c*, 1·9). Similar material was obtained by hydrogenation of ergosteryl acetate under similar conditions. 3β-Hydroxyergost-7-ene, prepared by alkaline hydrolysis of the acetate, formed needles, m. p. 149—150°, $[\alpha]_D$ −4° (*c*, 1·1). Barton and Cox (*loc. cit.*) give m. p. 148°, $[\alpha]_D$ −2° (*c*, 1·43).

3-*Ketoergost*-7-*ene*.—Oxidation of the foregoing sterol by the general chromic acid method gave this ketone as platelets from benzene, m. p. 158—159°, $[\alpha]_D$ +20° (*c*, 1·34) (Found : C, 84·4; H, 11·95. Calc. for $C_{28}H_{46}O$: C, 84·4; H, 11·6%). Barton and Cox (*loc. cit.*) give m. p. 159°, $[\alpha]_D$ +22° (*c*, 1·59).

Ergost-7-*ene*.—This was prepared by Kishner–Wolff reduction of the 3-ketone, as described for the preparation of ergosta-8(14)-ene. The hydrocarbon was purified by chromatography and by recrystallization from ethyl acetate–methanol. *Ergost*-7-*ene* exhibits "liquid crystal" formation when the m. p. is observed with crossed Nicol prisms, an anisotropic liquid forming at 83—84°, which becomes isotropic at 88—90°, $[\alpha]_D$ +0·5° (*c*, 1·15) (Found : C, 87·6; H, 12·8. $C_{28}H_{48}$ requires C, 87·4; H, 12·6%).

24-*Hydroxychol*-9(11)-*ene*.—Lithium aluminium hydride reduction of methyl chol-9(11)-enate {m. p. 65—66·5°, $[\alpha]_D$ +40° (*c*, 1·41)}, kindly supplied by Dr. Heymann, Harvard University, in ether at 0° gave, after chromatographic purification, a nearly quantitative yield of 24-*hydroxychol*-9(11)-*ene*, as needles [from light petroleum (b. p. 40—60°)], m. p. 103—104°, $[\alpha]_D$ +44° (*c*, 0·97) (Found : C, 83·45; H, 11·6. $C_{24}H_{40}O$ requires C, 83·6; H, 11·7%).

Methyl 3α-hydroxychol-9(11)-enate (m. p. 105—108°) was kindly provided by Professor C. W. Shoppee.

3β-*Hydroxyergost*-14-*ene*.—A sample of the acetate, which had m. p. 113—114·5°, $[\alpha]_D$ +11° (*c*, 2·39), was kindly supplied by Dr. D. H. R. Barton. Hydrolysis and recrystallization from methanol gave the sterol as plates, m. p. 142—143·5°, $[\alpha]_D$ +21° (*c*, 1·05). Barton and Cox (*loc. cit.*) record m. p. 141°, $[\alpha]_D$ +22° (*c*, 1·26). Hart and Emerson (*J. Amer. Chem. Soc.*, 1932, **54**, 1070) give m. p. 141°, $[\alpha]_D$ +21·2°.

Spectroscopic Technique.—All spectra were run on a Grubb–Parsons single-beam spectrometer as described previously (Barnard, Fabian, and Koch, *J.*, 1950, 2442). A lithium fluoride prism was used for the 3·3-μ. region. The following linear slit-widths were employed : 0·09—0·11 mm. at 3·3 and at 6 μ., 0·18—0·45 mm. at 10—15 μ. The accuracy of the frequency measurements given in the Table is estimated as ±5 cm.$^{-1}$ at 3·3 μ., ±3 cm.$^{-1}$ at 6 μ., and ±2 cm.$^{-1}$ at 10—15 μ.

The water-vapour concentration in the spectrometer was reduced by means of continuous air circulation through an external tower of activated alumina in a closed system (built for us by Messrs. Air Control Installations Ltd.), whereupon only the stronger H_2O absorption peaks near 6 μ. remained conveniently defined for calibration purposes as shown in Fig. 3. The broad energy minimum on the spectral traces, superimposed on the water-vapour bands above 1700 cm.$^{-1}$, is due to the peculiar characteristics (interference fringes) of the Bell thermistor receiver employed as radiation detector.

No attempt was made to apply micro-methods in this work, but the primary focus in the radiation unit of the spectrometer, where a reduced image of the Nernst filament source is formed, was found very suitable for work on a semi-micro-scale. Fixed araldite-sealed rock-salt cells of the type previously described (Barnard, Fabian, and Koch, *loc. cit.*) were used, requiring about 0·1 c.c. of solution. Under

Infra-red frequencies for steroid compounds containing nuclear tri- and tetra-substituted double bonds.

Compound.	3000—3100 cm.$^{-1}$ (3·3 μ.). (CCl_4 soln.) (sh. = shoulder).	790—850 cm.$^{-1}$ (12 μ.). (CS_2 soln.)(<20% A = w., 20—50% A = m., >50% A = st.).	1620—1700 cm.$^{-1}$ (6 μ.). ($CHCl_3$ soln.).
Hydrocarbons:			
(*a*) [Cholestane]	—	[w. 795, w. 827]	—
(*b*) Cholest-4-ene	sh. 3040	st. 810	1657
(*c*) Cholest-5-ene	weak 3030	st. 797, st. 832	1667
(*d*) Ergost-7-ene	sh. 3013, sh. 3040	m. 795, st. 825	1666
(*e*) Cholest-8(9)-ene	—	w. 800, w. 833	—
(*f*) Ergost-8(14)-ene	—	w. 797	—
Ketones:			
(*g*) [Cholestanone]	—	[w. 808]	—
(*h*) Ergost-7-en-3-one	sh. 3013, sh. 3040	st. 827, st. 847	~1664
(*i*) Cholest-8(9)-en-3-one	—	w. 815	—
(*j*) Ergost-8(14)-en-3-one	—	w. 812	—
Alcohols:			
(*k*) [Cholestanol]	—	[w. 795, w. 842]	—
(*l*) Cholesterol	weak 3030	st. 800, m. 840 (CS_2 or Nujol)	1669
(*m*) 3β-Hydroxyergost-7-ene	sh. 3020, sh. 3040	m. 792, st. 828, m. 847 (Nujol)	1664
(*n*) 3β-Hydroxycholest-8(9)-ene	—	m. 802, m. 847 (Nujol)	—
(*o*) 3β-Hydroxyergost-8(14)-ene	—	m. 845 (Nujol)	—
(*p*) 3β-Hydroxyergost-14-ene	strong 3055	m. 797, m. 807, st. 825	1648
(*q*) 24-Hydroxychol-9(11)-ene	strong 3042	m. 827, m. 852	1644
Esters:			
(*r*) Methyl chol-9(11)-enate	moderate 3037	[not measured]	1643
(*s*) Methyl 3-hydroxychol-9(11)-enate	moderate 3037	[not measured]	1645

the standard conditions chosen for this work, a total of about 60 mg. of sample was applied over the three characteristic spectral regions approximately as follows: 5 mg. dissolved in 0·15 c.c. of carbon tetrachloride, path-length 1 mm., at 3·3 μ.; 25 mg. (15—20 mg. for compounds containing >C=O) in 0·15 c.c. of chloroform, path-length 1 mm., at 6 μ.; 20 mg. in 0·1 c.c. of carbon disulphide, path-length 0·25 mm., at 10—15 μ. If so desired, the samples could be recovered from each solution, and a total of about 35 mg. would then suffice for all measurements. After recovery from chloroform, however, great care must be taken to remove the last traces of this solvent which has intense bands near 3·3 and 13 μ.

Some steroid substances containing free hydroxyl groups are not sufficiently soluble for these standard conditions. At 3·3 μ., if the sample is virtually insoluble in carbon tetrachloride, rather thick pastes with perfluorokerosene (Dupont) give quite serviceable spectral curves; at 6 μ., the chloroform solution path-length can be increased to about 3 mm. with fair success or, in favourable cases, the double-bond peak can also be discerned in relatively thick Nujol mulls; at 10—15 μ. Nujol mulls are applied in a thickness giving roughly the same overall appearance of the spectral intensities near 10 μ. as that obtained with the standard carbon disulphide solutions (cf. Fig. 2, l_1 and l_2). The quality of the spectra obtainable from Nujol mulls varies from sample to sample, depending on the microscopic behaviour of the particular crystal structure on being ground to a powder.

Results and Discussion.

The Olefinic C–H *Stretching Region* (3·3 μ.).—As may be seen from Fig. 1 and the Table of frequencies, the steroid hydrocarbons, ketones, and alcohols containing nuclear tetrasubstituted ($\Delta^{8(9)}$- or $\Delta^{8(14)}$-)double bonds resemble their saturated analogues in failing to give rise to selective absorption between 3000 and 3100 cm.$^{-1}$, reflecting the absence of olefinic C–H groups (cf. Jones *et al.*, *loc. cit.*, 1948). On the other hand, all nuclear trisubstituted ethylenic compounds exhibited such selective absorption to a greater or lesser degree (Fig. 1 and Table). The $\Delta^{9(11)}$- and Δ^{14}-groups gave quite well-defined bands, cholest-5-ene (*c*) and the corresponding alcohol, cholesterol (*l*), displayed small peaks, and the Δ^{4}- and Δ^{7}-compounds were just characterized by shoulders on the slope of the strong saturated C–H absorption; the shoulder was apparently double in the latter case. Only a single band or shoulder might perhaps be expected to arise in all these spectra, but both Fox and Martin (*loc. cit.*) and we have observed a doublet similar to that found for the Δ^{7}-compounds also in methyl*cyclo*hexene, presumably resulting from vibrational interaction of the single olefinic C–H bond with the neighbouring CH_2 group.

Clearly, the appearance of the 3000—3100-cm.$^{-1}$ spectral region examined under sufficient dispersion may help to distinguish between the tetrasubstituted ethylenic type on one hand,

and the trisubstituted (as well as other lesser substituted types) on the other. Some intensity differentiation between the various trisubstituted compounds also appears to take place and may perhaps be specific (see also below). Certain reserve must, however, be exercised in drawing structural conclusions on an unknown compound from this spectral region alone; *absence* of selective absorption may conceivably result from excessive weakness of the characteristic C–H band in a given case, and the *presence* of a band or shoulder must be checked against possible strong absorption near 1550 cm.$^{-1}$ which might give rise to a detectable first overtone. Unfortunately it is not possible to improve the prominence of weak bands by increasing sample concentration or optical resolution still further, as the persistence and effective resolution of these bands is limited by the overlapping of the adjoining relatively much more intense absorption region of saturated C–H just below 3000 cm.$^{-1}$.

The Olefinic C–H *Out-of-plane Bending Region* (10—15 μ.).—Similarly to the above, the presence or otherwise of olefinic C–H bonds in the steroid nucleus can frequently be recognized from the 10—15-μ. spectral region. All the trisubstituted ethylenic derivatives reported here displayed moderate or strong absorptions between 790 and 850 cm.$^{-1}$, where the tetrasubstituted examples, whether hydrocarbon, ketone, or alcohol, only gave relatively weak skeletal frequencies (Fig. 2 and third column of the table). The closest approach between the two types of unsaturation as examined under approximately standardized concentration conditions was that of the trisubstituted ethylenic 24-hydroxychol-9(11)-ene (*g*) which exhibited barely 40% absorption at 827 cm.$^{-1}$ and the tetrasubstituted 3β-hydroxycholest-8(9)-ene (*n*) which apparently (in Nujol) absorbed nearly 25% at 802 and 847 cm.$^{-1}$. It may also be noted here that the only substance giving rise to a moderately strong band between 650 and 700 cm.$^{-1}$ (the characteristic region of nuclear disubstituted double bonds) was cholest-4-ene (*b*).

With one exception (*b*), all the trisubstituted double-bond groups displayed *two* moderately strong bands instead of the single frequency normally expected for such systems at 790—850 cm.$^{-1}$. It may be that the true single frequency of the out-of-plane bending vibration of the olefinic C–H bond lies very near a weak skeletal vibration frequency of the same symmetry class which becomes greatly intensified through resonance interaction, so that two strong bands result. Such an explanation would be in accord with the weak bands generally observed in this spectral region for saturated and tetrasubstituted ethylenic steroid compounds.

The characteristic twin olefinic frequencies of cholesterol found at 800 and 840 cm.$^{-1}$ in either carbon disulphide solution (l_1) or the crystalline solid (l_2) are closely similar to those reported in the literature (*loc. cit.*). In view of the marked changes which occur in this spectral region of crystalline cholesterol, on cooling to very low temperatures (without, however, affecting the strong doublet) (Haines *et al.*, *loc. cit.*), the close correspondence between the absorption curves obtained at room temperature for the carbon disulphide solution and for the crystalline solid seems of interest. The physical condition of the steroid sample appears to have no important influence on the position or intensity of the diagnostic bands. Much more marked spectral changes at 790—850 cm.$^{-1}$ have been found to differentiate the various physical states of *cis*- and *trans*-polyisoprenes severally (Saunders and Smith, *loc. cit.*).

Diagnostic application of the C–H out-of-plane bending region has already been made in the structurally related pentacyclic triterpene group. Thus, two of us (quoted by Ames, Halsall, and Jones, *J.*, 1951, 450) inferred from the low absorption intensity of β-amyrene-III between 10 and 15 μ. that the double bond of this unsaturated hydrocarbon must be in a tetrasubstituted position. Similarly, Günthard (quoted by Dietrich, Meyer, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1950, **33**, 672, 711) found in the presence of a trisubstituted double bond in soyasapogenol-C reflected by a moderately strong band at 815 cm.$^{-1}$, and double-bond isomerization in a pentacyclic hydrocarbon was significantly correlated with the disappearance of a band at 800 cm.$^{-1}$ to give a high-transmission spectral curve. It would seem that extreme weakness of absorption in the 790—850-cm.$^{-1}$ range, examined at sufficient path-length or concentration, is a reliable criterion of the *absence* of trisubstituted double-bond groups; that bands of intermediate strength as observed in 3β-hydroxycholest-8(9)-ene or 24-hydroxychol-9(11)-ene may be diagnostically unreliable; and that strong bands as in cholesterol and cholest-4-ene give a fairly safe indication of the *presence* of such groups. Preliminary observations on disubstituted ethylenic compounds in the sterol group also lead us to conclude that weak absorption throughout the range 10—15 μ. may be regarded as proof of the absence of any but tetrasubstituted olefinic linkages in the steroid molecule.

The C≡C *Stretching Region* (6 μ.).—A reproduction of the actual spectral energy traces as obtained on the automatic recorder at 6 μ. is shown in Fig. 3 so as to afford an indication of the

slight degree of interference by water-vapour absorption and the relative prominence of the characteristic double-bond peaks which can be observed. The frequency position of these bands is given in the last column of the table. It was found that all of the trisubstituted ethylenic derivatives, including one unconjugated ketone (*h*) and two esters (*r* and *s*), displayed weak or moderate bands at 1640—1670 cm.$^{-1}$, whereas none of the saturated or tetrasubstituted olefinic compounds gave any such absorption under comparable conditions. Jones *et al.* (*loc. cit.*, 1950) similarly observed double-bond frequencies for ten Δ^5- (1669—1672), one Δ^7- (1664), four Δ^{14}- (1646—1648) and three Δ^{16}- (1621—1630 cm.$^{-1}$) unsaturated trisubstituted ethylenic molecules, but they failed to observe such a frequency in five different $\Delta^{9(11)}$- unsaturated steroids containing one or more carbonyl groups which render its detection rather difficult. The tetrasubstituted ethylenic cholest-8(14)-enol was also found transparent by these authors.

The diagnostically significant intensity differentiation observed between tri- and tetrasubstituted ethylenic steroids, in which the double-bond stretching vibration appears weakly allowed and forbidden respectively in the infra-red, is in accord with symmetry considerations. It seems unlikely that any of the bands found in this region should merely represent overtones of the C–H bending vibrations at 790—850 cm.$^{-1}$ rather than genuine double-bond stretching frequencies. Cholest-4-ene (*b*), for instance, displays a particularly intense characteristic frequency at 810 cm.$^{-1}$ which corresponds to a first overtone frequency at or below 1620 cm.$^{-1}$, whereas the observed 6-μ. absorption actually occurs at 1657 cm.$^{-1}$. Again, ergost-7-ene (*d*) exhibits a strong band at 825 cm.$^{-1}$ with a potential overtone normally expected at or below 1650 cm.$^{-1}$ so that the rather intense 1666-cm.$^{-1}$ peak is much more reasonably assigned to the stretching vibration of the double bond.

Only the Δ^5- and Δ^7-compounds give characteristic frequencies in the usual trialkylethylene range of 1660—1675 cm.$^{-1}$. These two appear too close together to permit unequivocal differentiation, although the Δ^5 frequencies seem consistently higher by a few wave-numbers. The Δ^{14}- and $\Delta^{9(11)}$-compounds (*f*) and (*q*, *r*, *s*) are similarly difficult to distinguish from each other on the basis of their double-bond vibration frequencies alone, but they are sharply differentiated from the other trialkylethylenes by the abnormal location of these bands below 1650 cm.$^{-1}$. On account of the relatively large shifts involved, we believe that changes from the normal stretching-force constants of trisubstituted double bonds must be at least partly responsible for the effect, rather than only unspecific changes in the effective mass of the vibrating groups. This supposition is supported by the following considerations.

The Effect of Steric Strain.—Monocyclic five-membered ring compounds containing one or more unsaturated carbon atoms, such as *cyclo*pentanone or *cyclo*pentene, are subject to appreciable steric strain in Baeyer's sense. Extrapolating the quantum-mechanical treatment of *cyclo*propane and *cyclo*butane by Coulson and Moffitt (*Phil. Mag.*, 1949, **40**, 1; cf. Förster, *Z. physikal. Chem.*, 1939, *B*, **43**, 58), such strain should be taken up by $s : p$ hybridization ratio changes in the carbon σ-valency orbitals and by formation of "bent" σ-bonds, resulting in all exocyclic bonds becoming slightly strengthened as well as the cyclic bonds being weakened. The theory accounts for the anomalous C–H bond force constants which are in fact observed in both *cyclo*propane and *cyclo*butane (*loc. cit.*), and corresponding force-constant changes may therefore be expected to occur also in strained unsaturated five-membered rings. The reality of such changes appears to be reflected in the unusually high stretching vibration frequencies of the olefinic C–H bonds in *cyclo*pentene (Kohlrausch and Seka, *Ber.*, 1936, **69**, 729; Kohlrausch, Seka, and Tramposch, *ibid.*, 1942, **75**, 1385) and of the carbonyl bond in *cyclo*pentanone (Hartwell, Richards, and Thompson, *J.*, 1948, 1036), as well as in the abnormally low C=C vibration frequencies of *cyclo*pentene and related hydrocarbons (Kohlrausch *et al.*, *loc. cit.*). We may conclude that the anomalous 1648-cm.$^{-1}$ frequency of 3β-hydroxyergost-14-ene (*p*) forms part of a more general phenomenon comprising also the related anomalies of Δ^{16}- and of 17-keto-steroids (Jones *et al.*, *loc. cit.*), and that it corresponds to a reduction in bond-stretching force constant resulting from σ-bond strain in ring D of the steroid nucleus. This interpretation receives further support from the unusually high (as well as abnormally intense) olefinic C–H stretching frequency of the Δ^{14}-molecule (*p*) at 3055 cm.$^{-1}$.

The lowered C=C stretching frequency of $\Delta^{9(11)}$-compounds (*q*, *r*, and *s*) finds a significant parallel in a similar anomaly of Δ^{11}-derivatives (Jones *et al.*, *loc. cit.*, 1950, and our observations). In both cases, space models suggest the possibility of appreciable steric strain resulting from *trans*-fusion of *cyclo*pentane and *cyclo*hexene rings. Related anomalies should also occur in $\Delta^{8(9)}$- and $\Delta^{8(14)}$-steroids but cannot be observed in the infra-red where the double-bond vibration is inactive. An interesting analogy seems to be provided, however, by the fusion of a *cyclo*butane to a *cyclo*hexene ring as in α-pinene, resulting in a reduction of the normal 1675-cm.$^{-1}$

double-bond stretching frequency of isomeric 1-methyl-4-*iso*propenyl*cyclo*hexene to 1656 cm.$^{-1}$ (A.P.I., *loc. cit.*).

If the apparent steric strain in both $\Delta^{9(11)}$- and Δ^{14}-steroids is assumed to be responsible also for a reduction in the strength of the π-bond of these ethylenic linkages, rather than for changes of σ-orbital hybridization alone, then the observed intensities of the adjacent C–H bond-vibration frequencies can be better understood. In the presence of σ-bond strain alone, we may expect a slight frequency increase and probably a negligible change of infra-red absorption intensity (cf. comparative extinction coefficients of sp^2 and sp^3 C–H bonds given by Fox and Martin, *loc. cit.*). For π-bond strain, on the other hand, involving reduced π-orbital overlap, the C–H bond frequency should remain essentially unchanged but its intensity might well be markedly affected on account of the greater residual electron density now remaining on the unsaturated carbon atom. Fig 1 illustrates the fact that a remarkable intensification of the olefinic C–H absorption occurs in 3β-hydroxyergost-14-ene (*p*) and to a lesser extent also in the $\Delta^{9(11)}$-compounds (*q*, *r*, and *s*).

It has been tacitly assumed in the foregoing that the C=C stretching vibration is entirely localized in the double bond and that interaction with neighbouring groups can be, as customary, neglected to a first approximation. This might no longer be true in the presence of steric strain, but the reported unusual infra-red absorption properties of the essentially localized olefinic C–H bond vibration strongly support the view that some real change from the normal electronic structure does occur at the unsaturated carbon atom.

Isolated double bonds in rings A or B of the steroid nucleus evidently do not create appreciable steric strain. Δ^5- and Δ^7-Groups display normal vibrational behaviour, and although the 1657-cm.$^{-1}$ frequency of cholest-4-ene (*b*) seems slightly low, it is not paralleled by any corresponding anomaly in the absorption of a Δ^2-group in the same ring (Jones *et al.*, *loc. cit.*, and forthcoming paper in this series).

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