218. *Steroids and Walden Inversion. Part XXVIII.* The Structure of the Lactonic Acid derived from* 6α-*Hydroxy*-2 : 3-seco*cholestane*-2 : 3-*dioic Acid.*

By D. R. James and C. W. Shoppee.

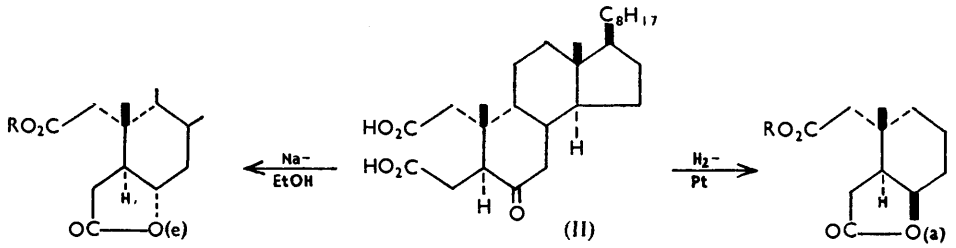
The lactonic acid, m. p. 202°, $[\alpha]_D$ −20°, derived from 6α-hydroxy-2 : 3-*seco*cholestane-2 : 3-dioic acid, is shown to be a 3→6α-γ-lactone (I; R = H) and not a 2→6α-δ-lactone (IV; R = H).

In Part VIII Shoppee and Summers [1] examined the capacity for lactonisation of the epimeric 6-hydroxy-2 : 3-*seco*cholestane-2 : 3-dioic acids. It was found that 6-oxo-2 : 3-*seco*-cholestane-2 : 3-dioic acid (II) by reduction with sodium–ethanol gave a compound regarded as the 3→6α-γ-lactonic acid (I; R = H), whilst hydrogenation with platinum in acetic acid or ethanol gave the 3→6β-γ-lactonic acid (III; R = H), convertible into the isomeride (I; R = H) by treatment with sodium ethoxide at 180°.

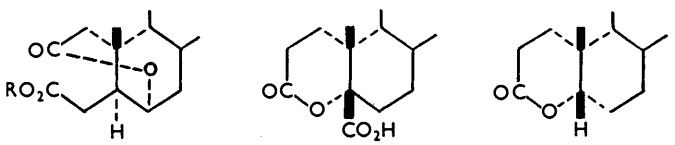
In a letter dated October 31st, 1952, Dr. A. Georg (Geneva) suggested that the evidence did not exclude a 2→6α-δ-lactonic acid structure (IV; R = H) for the compound, m. p. 202°, $[\alpha]_D$ −20°, and quoted as examples of stable steroid δ-lactones those of 5-hydroxy-3 : 4-*seco*cholestane-3 : 4-dioic acid [2] (V) and 5-hydroxy-3 : 5-*seco*-A-norcholestan-3-oic acid [3] (VI).

* Part XXVII, preceding paper.

[1] Shoppee and Summers, *J.*, 1952, 3374.
[2] Tschesche, *Ber.*, 1932, **65**, 185.
[3] Salamon, *Z. physiol. Chem.*, 1941—2, **272**, 61.



R	Formula	M. p.	$[\alpha]_D$	R	Formula	M. p.	$[\alpha]_D$
H	(I)	202°	−20°	H	(III)	196°	+58°
Me	(I)	99	−19	Me	(III)	99	+59



6α-Hydroxy-2 : 3-*seco*cholestane-2 : 3-dioic acid is not comparable with natural steroids because ring A has been broken, so that $C_{(5)}$, $C_{(6)}$, $C_{(7)}$, and $C_{(10)}$ have mobility comparable with that admitted for $C_{(1)}$, $C_{(2)}$, $C_{(3)}$, and $C_{(4)}$ in *trans*-decalin, and ring B can undergo strainless transition into a boat form. Examination of molecular models shows that there are two possible conformations of ring B as a boat form, that with ends at $C_{(6)}$ and $C_{(9)}$ (Fig. 1) and that with ends at $C_{(5)}$ and $C_{(8)}$ (Fig. 2). In both conformations 2→6β-δ-lactone formation with C–C and C–O bonds of normal length appears impossible, whilst 3→6β-γ-lactone formation involves only slight strain. In both conformations 3→6α-γ-lactone formation with bonds of normal length appears impossible, but in the second conformation (Fig. 2) a 2→6α-δ-lactone can be formed virtually without strain (Fig. 3).

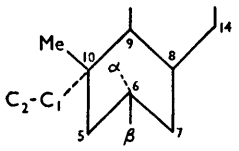


Fig. 1

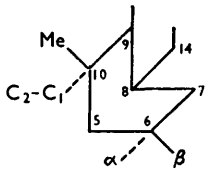


Fig. 2

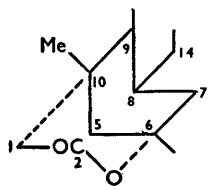
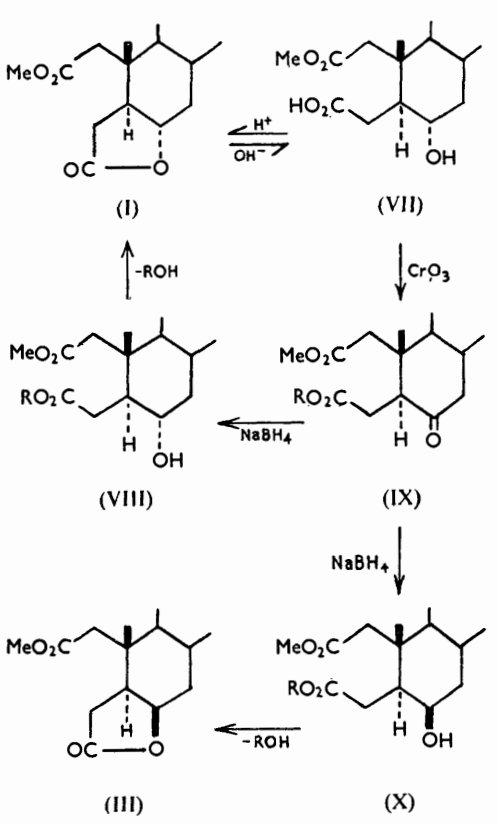


Fig. 3

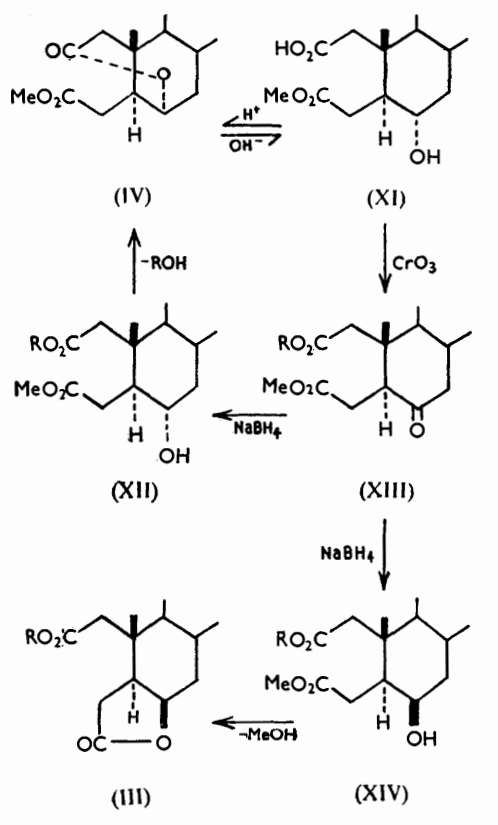
In view of the usual equilibrium between an acid and its lactone it might be possible for the slightly strained 3→6α-γ-lactonic acid (I; ring B in chair form) to pass into the strainless 2→6α-δ-lactonic acid (IV; ring B in boat form). It seemed desirable to attempt to ascertain the structure of the lactonic acid, m. p. 202°, $[\alpha]_D$ −20°, and it appeared possible to reach an experimental decision between formulæ (I) and (IV) in the following way.

If the lactonic methyl ester, m. p. 99°, $[\alpha]_D$ −19°, has structure (I; R = Me), treatment with one equivalent of sodium hydroxide should open the lactone ring, and oxidation of the resulting methyl hydrogen ester (VII) should yield 2-methyl 3-hydrogen 6-oxo-2 : 3-*seco*-cholestane-2 : 3-dioate (IX; R = H), convertible, *e.g.*, with diazoethane, into the 3-ethyl 2-methyl ester (IX; R = Et). Reduction of this ester with sodium borohydride should give 3-ethyl 2-methyl 6β-hydroxy-2 : 3-*seco*cholestane-2 : 3-dioate (X), possibly accompanied by the 6α-hydroxy-epimeride (VIII), which by spontaneous lactonisation should yield, with elimination of ethanol and methanol respectively, the *known* 3→6β-lactonic methyl ester (III; R = Me) and the starting material (I; R = Me).

If, however, the lactonic methyl ester, m. p. 99°, $[\alpha]_D$ −19°, has structure (IV; R = Me), ring fission with one equivalent of sodium hydroxide should give 3-methyl 2-hydrogen 6α-hydroxy-2 : 3-*seco*cholestane-2 : 3-dioate (XI), which, by oxidation should afford 3-methyl 2-hydrogen 6-oxo-2 : 3-*seco*cholestane-2 : 3-dioate (XIII; R = H), giving the unsymmetrical 2-ethyl 3-methyl ester (XIII; R = Et). Sodium borohydride reduction should



Scheme A



Scheme B

now furnish 2-ethyl 3-methyl 6β-hydroxy-2 : 3-*seco*cholestane-2 : 3-dioate (XIV; R = Et), with possibly some of the 6α-hydroxy-epimer (XII; R = Et), which by spontaneous lactonisation should yield, with elimination of methanol and ethanol respectively, the *unknown* 3→6β-lactonic ethyl ester (III; R = Et) and the starting material (IV; R = Me).

Experimentally, titration of the lactonic methyl ester (I or IV; R = Me) with one equivalent of sodium hydroxide at ~60° and treatment with potassium permanganate gave a product, m. p. 167—169°, $[\alpha]_D$ +12°, which was at first regarded as methyl hydrogen 6-oxo-2 : 3-*seco*cholestane-2 : 3-dioate (VII or XI); treatment with ethereal diazoethane gave a non-crystalline product, which by chromatography yielded only the starting material and by treatment with sodium borohydride and chromatography also furnished only the starting material. Attempts to prepare the lactonic ethyl esters (I or IV; R = Et) and (III; R = Et) by using ethereal diazoethane failed to give crystalline products, although by using the same diazoethane solution ethyl cholest-5-ene-3β-carboxylate,[4] m. p. 83°, was formed without difficulty. Finally, it was discovered that treatment of the lactonic methyl ester (I or IV; R = Me) with one equivalent of sodium hydroxide at ~60° for 5

[4] Marker, Oakwood, and Crooks, *J. Amer. Chem. Soc.*, 1936, **58**, 481.

min. and acidification in the cold with 2N-sulphuric acid gave the compound, m. p. 167—169°, $[\alpha]_D$ +12°. This is therefore 2-methyl 3-hydrogen (VII) or 3-methyl 2-hydrogen 6α-hydroxy-2 : 3-*seco*cholestane-2 : 3-dioate (XI), and appears to resist mild oxidation by permanganate; hydrolysis with hot ethanolic potassium hydroxide gave the lactonic acid (I or IV; R = H), m. p. 202°, $[\alpha]_D$ −20°, converted by ethereal diazomethane into the lactonic methyl ester (I or IV; R = Me), m. p. 99°, $[\alpha]_D$ −19°. Oxidation of the methyl hydrogen 6α-hydroxy-ester (VII or XI) with chromium trioxide–acetic acid gave methyl hydrogen 6-oxo-2 : 3-*seco*cholestane-2 : 3-dioate (IX or XIII; R = H), whose structure was confirmed by conversion with ethereal diazomethane into the dimethyl ester [5] (IX = XIII; R = Me). Esterification with (*a*) diazoethane and (*b*) *p*-bromophenacyl bromide gave the ethyl methyl ester (IX or XIII; R = Et) and the methyl *p*-bromophenacyl ester (IX or XIII; R = p-$C_6H_4Br{\cdot}CO{\cdot}CH_2$). Reduction of the ethyl methyl ester with sodium borohydride led to spontaneous lactonisation and furnished the 3→6β-lactonic methyl ester, m. p. 99°, $[\alpha]_D$ +57°, as the sole product; similar reduction of the methyl *p*-bromophenacyl ester gave 1-*p*-bromophenylethane-1 : 2-diol, and the3 →6β-lactonic methyl ester, m. p. 99°, $[\alpha]_D$ +57°.

The elimination of *p*-bromo-ω-hydroxyacetophenone, its recognition as its reduction product 1-*p*-bromophenylethane-1 : 2-diol, and the formation of the known 3→6β-lactonic methyl ester (III; R = Me) prove that the lactonic acid derived from 6α-hydroxy-2 : 3-*seco*cholestane-2 : 3-dioic acid is the 3→6α-γ-lactonic acid (I; R = H) and not the 2→6α-δ-lactonic acid (IV : R = H).

This chemical evidence can now be supported by physical evidence. The infrared absorption spectrum of the 3→6α-lactonic methyl ester (I; R = Me) in 1% solution in carbon disulphide showed bands at 1780 and 1162 cm.$^{-1}$ corresponding to the bands at 1780 and 1145 cm.$^{-1}$ exhibited in 1% carbon disulphide solution by the undoubted 3→6β-γ-lactonic methyl ester (III; R = Me). The peaks at 1780 cm.$^{-1}$ indicate a γ-lactonic structure, whilst the absorption bands at 1162 and 1145 cm.$^{-1}$ are probably associated with the carbon–oxygen linkages of the two γ-lactonic methyl esters. Since it is believed that the stretching frequency of the C–O linkage of an equatorial hydroxyl group is greater than that of a corresponding axial hydroxyl group,[6] the bands observed at 1162 and 1145 cm.$^{-1}$ confirm the 6α- and the 6β-configuration assigned to the compounds (I) and (III) respectively.

It thus appears that the slightly strained structure (I) containing ring B as a chair form possesses greater thermodynamic stability than the strainless structure (IV) containing ring B as a boat form (Fig. 3).

Experimental

For general experimental directions, see preceding paper. $[\alpha]_D$ are in $CHCl_3$ unless otherwise stated. Ultraviolet absorption spectra were determined in EtOH on a Unicam SP 500 spectrometer with corrected scale, and infrared absorption spectra in CS_2 on a Perkin-Elmer double-beam instrument.

Sodium Borohydride Reduction of Dimethyl 6-*Oxo*-2 : 3-seco*cholestane*-2 : 3-*dioate* (IX; R = Me).—A solution of sodium borohydride (30 mg.) in methanol was added to dimethyl 6-oxo-2 : 3-*seco*cholestane-2 : 3-dioate (120 mg.) in ether–methanol. The mixture was left at 15° for 2 hr., diluted, and extracted with ether. Working up in the usual manner afforded an oil (115 mg.) which was chromatographed on highly active neutralised aluminium oxide (4 g.) prepared in pentane. Elution with pentane to benzene–pentane (3 : 2) gave an oil (12 mg.). Further elution with benzene and benzene–ether (4 : 1) gave methyl 6β-hydroxy-2 : 3-*seco*-cholestane-2 : 3-dioate 3→6β-lactone (III; R = Me) (92 mg.), m. p. 99°, $[\alpha]_D$ +58°.

Treatment of the 3→6α- *and* 3→6β-*Lactonic Acids* (I, III; R = H) *with Diazoethane.*—To a solution of 6α-hydroxy-2 : 3-*seco*cholestane-2 : 3-dioic acid 3→6α-lactone (35 mg.) in ether was added excess of ethereal diazoethane. The solution was kept at 0° for 15 min. and the excess of diazoethane then destroyed by addition of 2N-hydrochloric acid. Working up in the usual way afforded an oily ethyl ester (36 mg.).

[5] Windaus, *Ber.*, 1903, **36**, 3752.
[6] Page, *J.*, 1955, 2017.

6β-Hydroxy-2 : 3-*seco*cholestane-2 : 3-dioic acid 3→6β-lactone (35 mg.) by similar treatment also furnished an oily ethyl ester (35 mg.).

The same treatment of cholest-5-ene-3β-carboxylic acid (30 mg.) gave ethyl cholest-5-ene-3β-carboxylate, m. p. 83°.

Methyl Hydrogen 6α-*Hydroxy*-2 : 3-seco*cholestane*-2 : 3-*dioate* (VII).—To a solution of methyl 6α-hydroxy-2 : 3-*seco*cholestane-2 : 3-dioate 3→6α-lactone (I; R = Me) (300 mg.) in dioxan (15 c.c.) was added 0·1N-sodium hydroxide (6·9 c.c.), and the mixture left at 60° for 5 min. The solution was cooled in ice and acidified with 2N-hydrochloric acid; extraction with ether, washing to neutrality with water, and evaporation gave *methyl hydrogen* 6α-*hydroxy*-2 : 3-seco-*cholestane*-2 : 3-*dioate* (VII) (302 mg.), m. p. 167—169°, $[\alpha]_D$ +12° (*c*, 0·8), after crystallisation from methanol [Found (after drying at 60°/0·01 mm. for 6 hr.): C, 72·8; H, 10·2. $C_{28}H_{48}O_5$ requires C, 72·4; H, 10·4%]. The infrared absorption spectrum of a Nujol mull showed bands at 1700, 1680, and 2600 cm.$^{-1}$ (CO_2H), 1735 and 1252 cm.$^{-1}$ (CO_2Me), and at 3260 cm.$^{-1}$ (bonded OH).

Methyl hydrogen 6α-hydroxy-2 : 3-*seco*cholestane-2 : 3-dioate (25 mg.) was refluxed in ethanolic 10% potassium hydroxide (5 c.c.) for 1 hr. The solution was diluted, acidified, and extracted with ether. The ether extract was washed to neutrality with water, dried, and evaporated. The residue by crystallisation from methanol gave 6α-hydroxy-2 : 3-*seco*cholestane-2 : 3-dioic acid 3→6α-lactone (I; R = H) (20 mg.), m. p. and mixed m. p. 200—202°, which with ethereal diazomethane gave methyl 6α-hydroxy-2 : 3-*seco*cholestane-2 : 3-dioate 3→6α-lactone (I; R = Me), m. p. and mixed m. p. 99°.

Methyl Hydrogen 6-*Oxo*-2 : 3-seco*cholestane*-2 : 3-*dioate* (IX; R = H).—Methyl hydrogen 6α-hydroxy-2 : 3-*seco*cholestane-2 : 3-dioate (VII) (230 mg.) was dissolved in acetic acid (35 c.c.), a 2% solution of chromium trioxide in acetic acid (1·9 c.c.) added, and the mixture left at 15° for 16 hr. Excess of chromium trioxide was destroyed by addition of methanol, and the solution diluted; extraction with ether and working up in the usual manner gave *methyl hydrogen* 6-*oxo*-2 : 3-seco*cholestane*-2 : 3-*dioate* (IX; R = H) (220 mg.), m. p. 122—123°, $[\alpha]_D$ +33° (*c*, 0·92), after crystallisation from pentane [Found (after drying at 60°/0·01 mm. for 6 hr.): C, 72·6; H, 10·1. $C_{28}H_{46}O_5$ requires C, 72·7; H, 10·0%].

Treatment of the 6-oxo-half-ester (20 mg.) with ethereal diazomethane and working up in the usual manner gave dimethyl 6-oxo-2 : 3-*seco*cholestane-2 : 3-dioate [5] (IX; R = Me) (21 mg.), m. p. and mixed m. p. 113—114° after crystallisation from acetone–methanol.

3-*Ethyl* 2-*Methyl* 6-*Oxo*-2 : 3-seco*cholestane*-2 : 3-*dioate* (IX; R = Et).—Methyl hydrogen 6-oxo-2 : 3-*seco*cholestane-2 : 3-dioate (IX; R = H) (100 mg.) in ether (10 c.c.) was treated with excess of ethereal diazoethane and left at 0° for 30 min. Excess of diazoethane was destroyed with dilute hydrochloric acid and the ethereal solution worked up in the usual manner to give 3-*ethyl* 2-*methyl* 6-*oxo*-2 : 3-seco*cholestane*-2 : 3-*dioate* (IX; R = Et) (105 mg.), m. p. 77—78°, $[\alpha]_D$ +22·5° (*c*, 0·67), after crystallisation from methanol [Found (after drying at 50°/0·01 mm. for 16 hr.): C, 72·8; H, 10·2. $C_{30}H_{50}O_5$ requires C, 73·4; H, 10·3%].

Sodium Borohydride Reduction of 3-*Ethyl* 2-*Methyl* 6-*Oxo*-2 : 3-seco*cholestane*-2 : 3-*dioate* (IX; R = Et).—Sodium borohydride (20 mg.), dissolved in methanol, was added to a solution of 3-ethyl 2-methyl 6-oxo-2 : 3-*seco*cholestane-2 : 3-dioate (80 mg.) in ether–methanol. The mixture was left at 15° for 2 hr., diluted, and extracted with ether. Working up in the usual manner afforded an oil (81 mg.), which was chromatographed on neutralised aluminium oxide (3 g.) prepared in pentane. Elution with pentane and benzene–pentane (3 : 7) yielded an oil (13 mg.). Elution with benzene–pentane (1 : 1; 3 × 10 c.c.) (7 : 3; 2 × 10 c.c.) gave material (59 mg.) which by crystallisation from pentane gave methyl 6β-hydroxy-2 : 3-*seco*cholestane-2 : 3-dioate 3→6β-lactone, m. p. and mixed m. p. 99°, as the sole crystalline product.

3-p-*Bromophenacyl* 2-*Methyl* 6-*Oxo*-2 : 3-seco*cholestane*-2 : 3-*dioate* (IX; R = *p*-$C_6H_4Br{\cdot}CO{\cdot}CH_2$).—A solution of methyl hydrogen 6-oxo-2 : 3-*seco*cholestane-2 : 3-dioate (IX; R = H) (126 mg.) in 0·1N-sodium hydroxide (2·9 c.c.) was made faintly acid (pH ~6·4) by addition of a few drops of 0·05N-hydrochloric acid. A solution of *p*-bromophenacyl bromide (75 mg.) in ethanol (8 c.c.) was added and the whole refluxed for 1 hr. Crystalline material (103 mg.) separated on cooling at 0° for 16 hr., which by recrystallisation from methanol gave 3-p-*bromophenacyl* 2-*methyl* 6-*oxo*-2 : 3-seco*cholestane*-2 : 3-*dioate* (IX; R = p-$C_6H_4Br{\cdot}CO{\cdot}CH_2$) as plates, m. p. 128—130°, $[\alpha]_D$ +12° (*c*, 0·96), $\lambda_{max.}$ 214 (log ε 3·91) and 258 mμ (log ε 4·06) [Found (after drying at 20°/0·01 mm. for 10 hr.): C, 65·65; H, 7·65. $C_{36}H_{51}O_6Br$ requires C, 65·55; H, 7·8%].

Reduction of 3-p-*Bromophenacyl* 2 *Methyl* 6-*Oxo*-2 : 3-seco*cholestane*-2 : 3-*dioate* (IX; R = *p*-C_6H_4 $Br{\cdot}CO{\cdot}CH_2$).—Sodium borohydride (30 mg.), dissolved in methanol, was added to a solution

of the 3-*p*-bromophenacyl 2-methyl ester (82 mg.) in ether–methanol. The mixture was left at 15° for 2 hr., diluted, and extracted with ether. Working up in the usual manner afforded an oil (75 mg.), which by trituration with pentane gave a sticky solid; the pentane was decanted off and the residual solid washed several times with pentane to give crystalline material (20 mg.). Recrystallisation from methanol gave 1-p-*bromophenylethane*-1 : 2-*diol*, m. p. **100—101°**, $\lambda_{max.}$ 220 (log ε 4·03) and 265 mμ (log ε 2·6) [Found (after drying at 20°/0·01 mm. for 12 hr.) : C, 44·15; H, 4·35. $C_8H_9O_2Br$ requires C, 44·25; H, 4·2%]. The pentane washings were collected and chromatographed on neutralised aluminium oxide (3 g.) prepared in pentane. Elution with pentane and benzene–pentane (1 : 4; 2 × 8 c.c.) gave an oil (5 mg.). Elution with benzene–pentane (1 : 3; 2 × 8 c.c.)—(1 : 1; 2 × 8 c.c.) gave an oil (38 mg.) (negative Beilstein reaction), which by crystallisation from pentane afforded methyl 6β-hydroxy-2 : 3-*seco*cholestane-2 : 3-dioate 3→6β-lactone, m. p. and mixed m. p. 99°, $[\alpha]_D$ +57°, as the sole crystalline product.

One of us (D. R. J.) acknowledges a grant from the D.S.I.R.; we thank Glaxo Laboratories Ltd. for a gift of cholesterol and for measuring the infrared absorption spectra.

UNIVERSITY OF WALES, UNIVERSITY COLLEGE, SWANSEA. [*Received, September 16th*, 1955.]