204. *An Improved Apparatus for the Microhydrogenation of Organic Compounds.*

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THE investigations now being carried out in these laboratories on the lipochromes and other unsaponifiable constituents of different species of marine life (Burkhardt *et al.*, *Biochem. J.*, 1934, **28**, 1698; Heilbron, Jackson, and Jones, *ibid.*, 1935, **29**, 1384; Heilbron and Phipers, *ibid.*, p. 1369) have necessitated the construction of an apparatus suitable for quantitative microhydrogenation.

The first account of such an apparatus is due to Hyde and Scherp (*J. Amer. Chem. Soc.*, 1930, **52**, 3359), who adapted the Warburg respirometer ("Über den Stoffwechsel der Tumoren," Berlin, 1926) for this purpose. A further modification was introduced by Kuhn and Möller (*Angew. Chem.*, 1934, **47**, 145), who applied a differential principle in which the difference between the volume of hydrogen absorbed by a known weight of a control substance and by the experimental substance was measured. This apparatus is very sensitive to small temperature changes and requires to be operated in an accurate thermostat.

Smith (*J. Biol. Chem.*, 1932, **96**, 35) developed an "absolute" method, in which the pressure of hydrogen in the apparatus is maintained constant, and the diminution in volume due to absorption is measured directly. By employing a reaction vessel of suitable design, effects of temperature were largely eliminated and the apparatus functioned without thermostatic control.

The design of a suitable apparatus is complicated by the fact that the reaction vessel must be vigorously shaken to cause hydrogenation. Kuhn and Möller (*loc. cit.*) rocked the whole apparatus, including the measuring system, but Smith (*loc. cit.*) agitated the reaction vessel separately, the necessary freedom of motion being supplied by a coil of glass tubing connecting the reaction system to the burette. Further, consequent upon the necessity of shaking, the method of introducing the substance to be hydrogenated offers some difficulty. It is essential that complete equilibrium between catalyst and hydrogen atmosphere be attained before the reaction commences; this requires considerable agitation, whilst the weighed quantity of material must be introduced afterwards without simultaneous entry of air.

In the present apparatus we have modified Smith's method as regards shaking, have devised an improved means of introducing the substance, and have also made complete compensation for temperature changes.*

EXPERIMENTAL.

The apparatus is illustrated diagrammatically in Fig. 1. The reaction system R is clamped to a wooden framework † arranged to swing about an axis through the hollow ground-glass bearing b_1b_2 (No. 1, size of ground joint) kept in position by steel springs. Through the centre of the bearing, communication is made with the mercury burette B (of 4 ml. capacity, graduated in 0·01 ml.). The height of the mercury reservoir is adjusted by means of a mechanical device which permits the burette level to be accurately controlled. Included in the system is a mercury manometer m and a McLeod gauge, which enables the presence of any leak to be readily detected. The system is evacuated with an oil pump at E, and pure dry hydrogen is admitted through the tap 1. The capillary tubing used throughout is of 1·5 mm. bore.

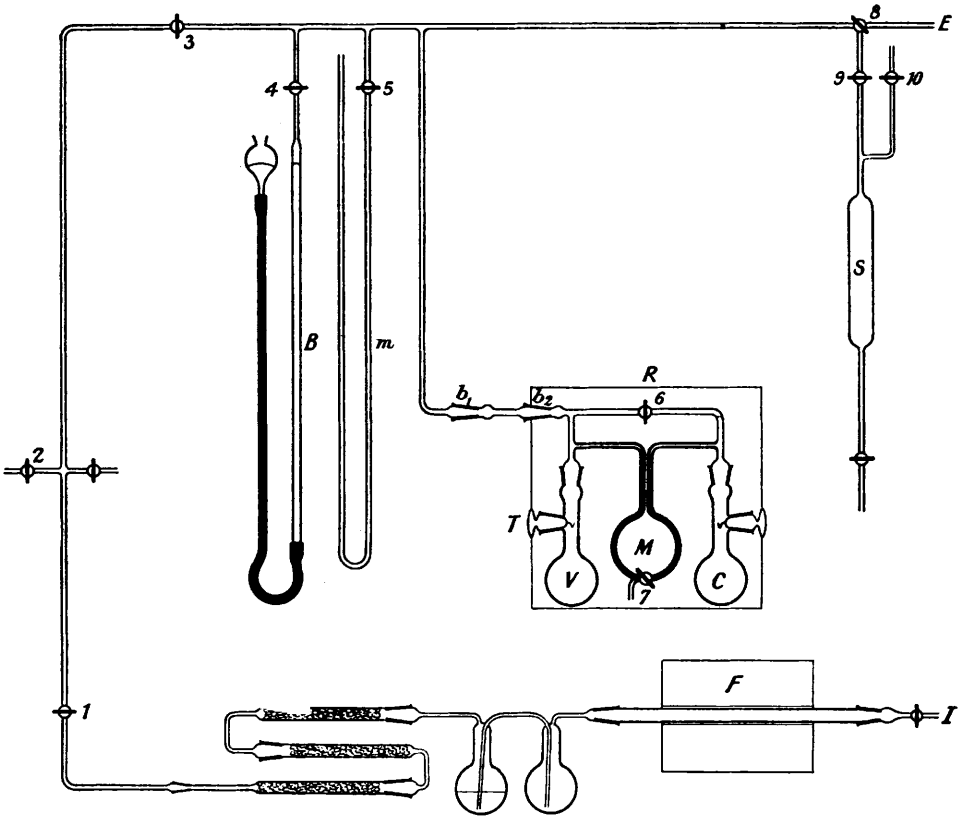
The Reaction System R.—This consists of a reaction vessel V (diameter of bulb, 4·5 cm.; neck 1·75 × 14 cm.) and a compensator C connected by ground-glass joints (No. 2 size) to

* There has recently come to our notice a paper by Slotta and Blanke (*J. pr. Chem.*, 1935, **143**, 3) in which another modification of Smith's apparatus is described; this differs in many important features from our own.

† This is supported by two light "Meccano" towers screwed rigidly to a heavy baseboard. The lower end of the shaker is coupled directly to a geared motor (ratio 14 : 1) by an eccentric which allows motion of the reaction system through an arc of some 20°. A variable resistance is included in the motor circuit.

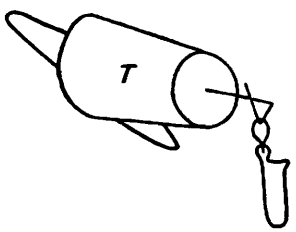
the arms of a capillary manometer containing *n*-butyl phthalate, as recommended by Smith (*loc. cit.*). At the lower extremity of the manometer is a three-way tap, 7, which facilitates introduction of the manometric fluid and, in conjunction with tap 6, permits the compensator system to be isolated from the reaction system. Into the side of the reaction vessel is fused a

FIG. 1.



ground-glass tap *T* (1·5 cm. internal diam.) at the extremity of which a stout platinum wire is attached bent at the end so as to form a hook (see Fig. 2). The correct shaping of this hook is of great importance for the efficient operation of the dropping mechanism. The substance to be hydrogenated is contained in a small glass tube (10 × 5 mm.) supported from the hook by a platinum loop as shown. At the desired moment the tube (which can be used indefinitely) is dropped into the flask by turning the tap in a clockwise direction.

FIG. 2.



Temperature Compensation.—In Smith's apparatus the influence of temperature fluctuations upon the gaseous volume of the reaction vessel was compensated for by means of an exactly similar flask, no account being taken of the volume of the remainder of the apparatus including the manometer and the connections thereto. We have not found this sufficiently accurate when temperature changes of the order of a few degrees occur, and accordingly we have measured the *total* volume of the apparatus and increased the volume of the compensator flask *C* so as to make compensation complete. As so designed, small erratic fluctuations of the *n*-butyl phthalate manometer were encountered, due to differential temperature effects caused by air currents. These were completely eliminated by enclosing the reaction system, including burette and shaking mechanism, in a draught-proof wooden chamber provided with a door and window through which the necessary observations are made.

Determination of the Volume of the Apparatus.—This was carried out by allowing a known volume of air, enclosed in the calibrated vessel *S*, to expand into the evacuated system (taps 3 and 4 being closed) by opening taps 8 and 9. The resultant pressure was measured on the mercury manometer *m*, and the required volume calculated by application of Boyle's law. Closure of taps 6 and 7 and repetition of the experiment enabled the volume of the reaction system, exclusive of the compensating system, to be separately determined; the volume of the latter is then found by difference, and its volume increased (by enlargement of the flask bulb) so as to become equal to that of the former.

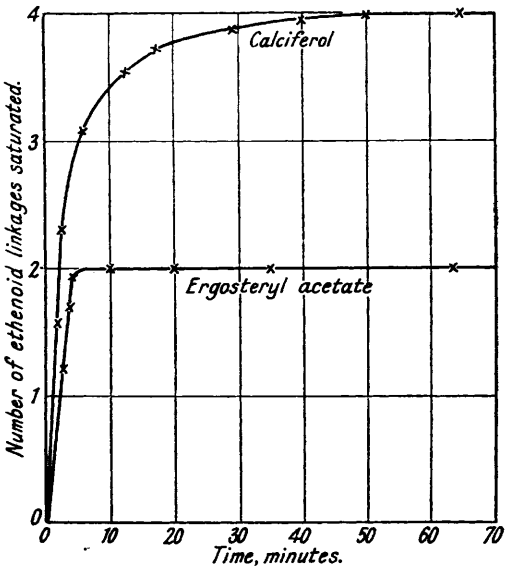
Reagents.—(a) *Hydrogen.* High-quality cylinder hydrogen (supplied by The British Oxygen Co. Ltd.) was purified before use by passage over platinised asbestos heated to 600° in the electric furnace *F*, followed by washing with sodium plumbite solution (Kuhn and Möller, *loc. cit.*) and drying over calcium chloride. It will be observed (Fig. 1) that throughout the purification train, ground-glass joints were used, lubricated with " apiezonfett " (Grade L).

(b) *Solvents.* Glacial acetic acid (A; see table) and decalin (D) or mixtures of these (D : A) were employed. The acetic acid was kept for a few days over chromic anhydride and then distilled twice. The decalin was shaken with 10% fuming sulphuric acid, left for 2 days, and the colourless upper layer then removed, washed, dried, and distilled.

(c) *Cleaning agents.* The ground-glass joints of the reaction vessel and the glass bearings were cleaned with thiophen-free toluene.

(d) *Catalysts.* Adams's platinic oxide, platinum-black deposited on alumina or barium sulphate, and palladium-black adsorbed on barium sulphate were used. The metallic catalysts were prepared by reduction of the chloride with sodium formate, and stored in an evacuated desiccator over solid potassium hydroxide.

FIG. 3.



Procedure.—Before each determination, taps 6 and 7 were closed and vessels *V* and *C* disconnected. After removal of grease with toluene, the flasks were cleansed by washing with soap and hot water, rinsed in glass-distilled water, and dried at 110°. The glass bearing b_1b_2 was cleaned and regreased after each determination. The catalyst was introduced into the reaction vessel in a small open glass tube and followed by 2 c.c. of solvent in each flask. The tap *T* was next inserted, the tube containing the weighed substance lowered down the neck of the vessel by means of a hooked wire, and attached to the platinum hook by suitable manipulation of the tap. The reaction vessel and compensator were fitted in place, the levelling tube of the burette closed with a clip (taps 1, 2, and 9 closed; 3, 4, 5, and 6 open), and the apparatus slowly evacuated. Tap 8 was now closed, and hydrogen admitted until a slight excess pressure of 20—30 mm. was registered on manometer *m* (10 mins.). This procedure was repeated three times.

Taps 3 and 5 were now closed, tap 7 opened, the clip removed from the burette, and the shaker set in motion. Immediate absorption of hydrogen commenced, as indicated by displacement of the levels of the *n*-butyl phthalate manometer when tap 6 was closed. The necessity for constant observation during this process was avoided by closing tap 7; the apparatus could then be left running, and the accumulated pressure differences at equilibrium balanced out in one operation.

When no further absorption occurred, the shaker was stopped, and the apparatus left until the levels of the phthalate manometer remained constant when tap 6 was closed. The pressure was next lowered to atmospheric by opening taps 2, 3, and 6, after which these taps were closed, and readings taken of the burette level, barometric pressure, and temperature of the enclosure. The tube containing the substance was now released, shaking recommenced, and the burette readings taken after given intervals of time by levelling the phthalate manometer. When no further absorption occurred, shaking was stopped, and the apparatus allowed to stand

until a constant burette reading was again obtained. The volume of hydrogen absorbed was reduced to *N.T.P.*, and the number of double bonds calculated accordingly.

During the hydrogenation of a few substances, the hydrogen uptake at room temperature did not correspond to an integral number of double bonds. In these cases, closing tap 7 and surrounding the reaction flask with a small beaker containing hot water (90°) for a few minutes, followed by shaking to a cold equilibrium, increased the hydrogen uptake to a satisfactory value. Blank determinations on catalyst alone, and on substances which underwent hydrogenation to completion in the cold, have demonstrated that the equilibrium between catalyst and hydrogen is entirely unaffected by such treatment.

Results.—The table summarises the results of determinations carried out on compounds of widely varying nature. Those substances which have been subjected to heat treatment are marked with an asterisk, whilst the figures in parentheses denote the corresponding " cold " value. The time of hydrogenation is not included, since it varies considerably with the weights of substance hydrogenated and of catalyst used; in the recorded determinations the time required varied from a few seconds up to one hour. Generally about 10 mg. of Adams's platinic oxide or 50 mg. of surface catalyst were employed; no advantage is to be gained by reducing the amount of catalyst, for the time of hydrogenation is merely prolonged. Graphical representation of the rate of hydrogen uptake of different substances enables useful comparisons to be made. An example of this is given in Fig. 3, where comparative rates of hydrogenation of calciferol and ergosteryl acetate are shown.

The applicability of the method to the hydrogenation of liquids of low vapour pressure is also illustrated in the table. In this respect it would appear that our mechanism for the introduction of the substance under examination possesses some advantage over those previously described, where a sealed capsule is used.

Table of Results.

Substance.	Formula.	Mol. wt.	Wt. hydrogenated, mg.	Solvent.†	Catalyst.	H_2 absorbed at N.T.P., c.c. Calc.	Found.	No. double bonds. Theory.	Found.	Ref.
Crotonic acid.....	$C_4H_6O_2$	86·0	4·445	D : A	$Pt/BaSO_4$	1·12	1·16	1·0	1·0	
,, ,, ...	,,	,,	6·374	D : A	$Pt/BaSO_4$	1·66	1·66	1·0	1·0	
Naphthalene	$C_{10}H_8$	128·0	3·666	A	$Pt/BaSO_4$	3·21	3·21	5·0	5·0	
Cinnamic acid ...	$C_9H_8O_2$	148·0	4·720	A	$Pt/BaSO_4$	2·86	2·86	4·0	4·0	
Ergosteryl acetate	$C_{30}H_{46}O_2$	438·4	9·983	D : A	$Pt/BaSO_4$	1·53	1·09	3·0	2·1	
,, ,,	,,	,,	9·742	D : A	PtO_2	1·50	1·05	3·0	2·1	
Lumisteryl acetate	$C_{30}H_{46}O_2$	438·4	9·920	D : A	Pt/Al_2O_3	1·52	1·46	3·0	2·9* (2·7)	1
,, ,,	,,	,,	9·580	D : A	$Pt/BaSO_4$	1·45	1·49	3·0	3·0* (2·7)	
Calciferol	$C_{28}H_{44}O$	396·4	10·610	D : A	$Pt/BaSO_4$	2·40	2·40	4·0	4·0	
Lumistadiene-triol diacetate	$C_{32}H_{50}O_5$	514·4	12·579	D : A	Pt/Al_2O_3	1·10	0·60	2·0	1·1	1
Lumistatrienone	$C_{28}H_{42}O$	394·4	5·777	A	PtO_2	1·31	1·27	4·0	3·9* (3·2)	
Lupeol acetate ...	$C_{32}H_{52}O_2$	468·4	11·020	A	Pt/Al_2O_3	0·53	0·54	1·0	1·0* (1·0)	2
β-Carotene.....	$C_{40}H_{56}$	536·5	1·582	D : A	PtO_2	0·73	0·73	11·0	11·0	2
,, ...	,,	,,	6·791	D : A	Pt/Al_2O_3	3·12	3·18	11·0	11·2	3
α-Carotene.....	$C_{40}H_{56}$	536·5	1·470	D : A	PtO_2	0·68	0·68	11·0	11·0	3, 4
Astacene	$C_{40}H_{48}O_4$	592·4	2·490	A	Pt/Al_2O_3	1·22	1·23	15·0	13·0* (11·2)	5, 6
Fucoxanthin	$C_{40}H_{60}O_6$	632·4	9·570	A	PtO_2	—	3·01	—	8·9	7
,, ...	,,	,,	2·012	A	PtO_2	—	0·63	—	8·9	
Sulcatoxanthin ...	$C_{40}H_{52}O_8$	660·5	1·382	A	PtO_2	—	0·42	—	9·0	8
,, ...	,,	,,	2·009	D : A	PtO_2	—	0·62	—	9·0	
Methyl azafrin ...	$C_{28}H_{40}O_4$	490·4	4·694	A	Pt/Al_2O_3	1·67	1·70	7·0	7·1	9
1-Keto-7-methoxy-1 : 2 : 3 : 4 : 9 : 10-hexahydrophenanthrene	$C_{15}H_{16}O_2$	228·1	3·110	A	PtO_2	—	1·86	—	6·1	10
			1·370	A	PtO_2	—	1·37	—	5·9	
			2·262	A	$Pd/BaSO_4$	—	0·46	—	2·1	
n-Butyl phthalate	$C_{16}H_{22}O_4$	278·0	5·045	D : A	PtO_2	1·22	1·22	3·0	3·0	

† See p. 897.

[1] Heilbron, Spring, and Stewart, J., 1935, 1221. [2] Zechmeister, Cholnoky, and Vrabély, *Ber.*, 1928, **61**, 566. [3] Kuhn and Möller, *loc. cit.* [4] Smith, *J. Biol. Chem.*, 1933, **102**, 157. [5] Karrer and Loewe, *Helv. Chim. Acta*, 1934, **17**, 745. [6] Karrer, Loewe, and Hübner, *ibid.*, 1935, **18**, 96. [7] Heilbron and Phipers, *loc. cit.* [8] Heilbron, Jackson, and Jones, *loc. cit.* [9] Kuhn, Winterstein, and Roth, *Ber.*, 1931, **64**, 333. [10] Robinson and Schlittler, J., 1935, 1285.

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