TABLE I[a]

MOLECULAR ROTATION DIFFERENCES (Δ^{Ac}) FOR 17-HYDROXY-20-KETOSTEROIDS

No.	Compound	M_D 17-Alcohol	17-Acetate	Δ^A
1	3β,17α-Dihydroxy-5-pregnen-20-one[b,c]	−114 Di	−254 Di	−140
2	3β,17α-Dihydroxy-5-pregnen-20-one 3-monoacetate[c,d]	− 94 Di	−271 Di	−177
3	17α-Hydroxy-4-pregnene-3,20-dione[c,d]	+354 Di	+208 Di	−146
4	3β,17α-Dihydroxyallopregnan-20-one 3-monoacetate[c,d]	+ 71 Di	− 39 Di	−110
5	3α,17α-Dihydroxypregnane-11,20-dione 3-monoacetate[e,f]	+327 An	+202 Chf	(−125)
6	Methyl 3α-acetoxy-17α-hydroxy-11-ketoetiocholane-17-carboxylate[f,g]	+289 Chf	+ 98 Chf	−191
7	3α,17α,21-Trihydroxypregnane-11,20-dione 3,21-diacetate[e,f]	+416 An	+201 Chf	(−215)
8	17α,21-Dihydroxypregnane-3,11,20-trione 21-monoacetate[e,f]	+333 An	+123 Chf	(−210)
9	17α,21-Dihydroxyallopregnane-3,11,20-trione 21-monoacetate[e,f]	+406 Chf	+197 Chf	−209
10	17α,21-Dihydroxy-4-pregnene-3,20-dione 21-monoacetate[c,d]	+512 Di	+213 Di	−299
11	17α,21-Dihydroxy-4-pregnene-3,11,20-trione 21-monoacetate[c,d]	+852 Di	+591 Di	−261
12	3β,17β-Dihydroxyallopregnan-20-one 3-monoacetate[d,h]	−120 Di	+ 6 Di	+126
13	3β,17β-Dihydroxy-5-pregnen-20-one[i,j]	−199 Chf	−198 Di	(+ 1)
14	3β,17β-Dihydroxy-5-pregnen-20-one 3-monoacetate[i,k]	−229 Chf	−225 Di	(+ 4)

[a] An = acetone, Chf = chloroform, Di = dioxane. Figures in the last column that are enclosed in parentheses indicate determinations made in different solvents. [b] P. L. Julian, E. W. Meyer and I. Ryden, THIS JOURNAL, **72**, 367 (1950). [c] R. B. Turner, *ibid.*, **75**, 3489 (1953). [d] R. B. Turner, this investigation. [e] L. H. Sarett, THIS JOURNAL, **70**, 1454 (1948). [f] Huang-Minlon, E. Wilson, N. L. Wendler and M. Tishler, *ibid.*, **74**, 5394 (1952). [g] E. Wilson and M. Tishler, *ibid.*, **74**, 1609 (1952). [h] C. W. Shoppee and D. A. Prins, *Helv. Chim. Acta*, **26**, 185 (1943). [i] C. W. Shoppee and D. A. Prins, *ibid.*, **26**, 201 (1943). [j] L. Ruzicka, M. W. Goldberg and F. Hunziker, *ibid.*, **22**, 707 (1939). [k] L. Ruzicka and H. F. Meldahl, *ibid.*, **21**, 1760 (1938).

which differs from other members of the series in the nature of the carbonyl function. The shift in molecular rotation occasioned by acetylation of 17β-hydroxy-20-keto derivatives (Nos. 12, 13 and 14), on the other hand, is either small or in a *positive* direction. The Δ values recorded for compounds 13 and 14, both of which possess 5,6-unsaturation, are zero, within the limits of the method. Although this result may be significant, it should be noted that the optical measurements in these cases were obtained in different solvents and hence are less reliable than those for which the same solvent was employed.

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Preparation of Crystalline Anhydrous β-Gentiobiose

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Crystalline anhydrous β-gentiobiose has heretofore been difficult to obtain because of crystallization difficulties. Consequently, the more easily crystallized but unstable bis-(methyl alcoholate), an α-D form, is usually prepared. We wish to report herein an improved procedure for the preparation of crystalline anhydrous β-gentiobiose based upon the finding that this crystalline phase forms quite readily at elevated temperatures. A similar temperature effect has been noted in the crystallization of the trisaccharide panose, 4-α-(isomaltopyranosyl)-D-glucose.[2]

Both of the above-mentioned crystalline forms of gentiobiose were first obtained by Bourquelot and Hérissey,[3] who reported for the crystalline anhydrous form, the constants: m.p. 190–195°, $[\alpha]_D$ −6° (6 min.) → +9.8° (final). Hudson[4,5] calculated that the initial specific rotation of β-gentiobiose should be −11°. Extrapolation of Bourquelot and Hérissey's data to zero time gives a value near −11°. For our preparation we find the constants: m.p. 188–190° (cor.), $[\alpha]^{25}_D$ −3.0° (initial, extrapolated) → +10.5° (final, *c* 4, water). This divergence of 8° between the calculated and determined rotations for β-gentiobiose appears to be real. It is interesting to compare it with the determined value of +166° (calculated[4] +175°) found by Fletcher and Diehl[6] for the related (1 → 6)-linked disaccharide α-melibiose. That the crystalline form of gentiobiose herein described is a molecular compound containing a small amount of the α-anomer is possible but no evidence of this could be obtained.

It is convenient to isolate and purify gentiobiose, regardless of its source, in the form of its β-octaacetate because of the fine crystallizing properties of this substance. One of the better sources of this sugar is hydrol (the mother liquor or "molasses" from the commercial production of α-D-glucopyranose monohydrate) from which it can be isolated as the acetate by the method of Berlin.[7] We consider the presently described directions as the procedure of choice for preparing gentiobiose should the α-D form not be required.

Experimental

β-Gentiobiose.—β-Gentiobiose octaacetate[7] (15 g.) was suspended in 180 ml. of 0.05 *N* sodium methoxide in dry methanol and allowed to stand, with occasional shaking, at room temperature for 1 hr. It was then diluted with water to dissolve the suspended material and passed through columns of Amberlite IR-120[8] and Duolite A-4.[9] The re-

(1) Corn Industries Research Foundation Associate.

(2) S. C. Pan, L. W. Nicholson and P. Kolachov, THIS JOURNAL, **73**, 2549 (1951).

(3) E. Bourquelot and H. Hérissey, *Compt. rend.*, **135**, 290 (1902); *J. pharm. chim.*, [6] **16**, 417 (1902).

(4) C. S. Hudson, THIS JOURNAL, **38**, 1566 (1916).

(5) C. S. Hudson, *ibid.*, **46**, 483 (1924).

(6) H. G. Fletcher, Jr., and H. W. Diehl, *ibid.*, **74**, 5774 (1952).

(7) H. Berlin, *ibid.*, **48**, 2627 (1926); F. J. Bates and Associates, "Polarimetry, Saccharimetry and the Sugars," Circular of the Natl. Bur. Standards C440, 1942, p. 463.

(8) A product of Rohm and Haas Co., Philadelphia, Pa.

(9) A product of the Chemical Process Co., Redwood City, Calif.

sultant solution was evaporated to a sirup under reduced pressure and the residual water was removed by repeated distillation with 100% ethanol under reduced pressure. The sirup was then dissolved in 40 ml. of hot methyl cellosolve (ethylene glycol monomethyl ether), filtered, nucleated and placed in an oven at 80° overnight. The crystalline material was filtered and washed with 100% ethanol; yield 6.7 g. (89%), m.p. 187–189° (cor.), $[\alpha]^{28}D$ −1.5° (initial, extrapolated) → +10.6° (final, *c* 4, water). The material was further purified by recrystallization from methyl cellosolve; yield 6.4 g. (95%), m.p. 190° (cor.), $[\alpha]^{25}D$ −3.0° (initial, extrapolated) → +10.5° (final, *c* 4, water). These constants were unchanged on further crystallization from this solvent or from ethanol effected by solution in minimal water and addition of warm 100% ethanol followed by crystallization at 60° (an ethyl alcoholate was not formed). The constants were likewise unaltered on standing under 95% ethanol (twice changed) for 1 week.

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Dibenzofuran-2-sulfonic and 3-Nitro-8-sulfonic Acids as Reagents for Amines and Amino Acids[1]

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Dibenzofuran-2-sulfonic acid, recently prepared in this Laboratory[2] is a strong acid and reacts with most amines to give stable salts. Simple salt formation from aqueous solution is much more convenient for preparation of derivatives than reactions commonly employed to identify amines such as acylation, quaternary base formation, etc. We have found that dibenzofuran-2-sulfonic acid (I) precipitates a large variety of amines and amino acids and that the salts formed are crystallizable solids having good melting points. The saturated solution of I in water is 4 *N* at 111° (99 g. of acid per 35 g. of water) and 0.34 *N* at 25°.

Since 3-nitrodibenzofuran has long been known,[3,4] it was expected that the sulfonic acid obtained from it would be an interesting and useful variant of compound I, and might have value in identifying amino compounds for which I was not suited. However, our expectations were not confirmed. The new compound, presumably the 3-nitro-8-sulfonic acid of dibenzofuran (compound II), was successfully prepared[5] but with difficulty, and it has none of the favorable crystallization characteristics of compound I. The free acid, II, appears to be very soluble in water from which it cannot be separated by usual crystallization methods. Crystallization from acetic acid however was satisfactory and gave the desired product in suitable purity. Despite its water solubility, II precipitates rapidly by salt formation with various amines, particularly aromatics. However, many of these only decomposed at high temperatures without showing characteristic melting points, hence have much less promise as derivatives than the salts from I.

The amino acid derivatives of dibenzofuran-2-sulfonic acid had been previously prepared and reported by Wendland and Smith.[6] We wish to add to those results the present observation that the neutral equivalents determined in alcoholic solution by the Foreman method[7] are highly reliable, with variations mostly ±1 from calculated and at worst only ±3. Thus this analytical procedure is most valuable in amino acid identifications. The salt of a monoamino monocarboxy acid titrates as a dibasic acid, a diamine monocarboxy acid as a tribasic acid, etc. The same titration can probably be applied also to the simple amine salts.

Experimental Part

Amino Salts of Dibenzofuran-2-sulfonic Acid.—The acid was prepared according to the method described in (2). The purified amine (1 to 2 g.) was mixed with sufficient water to effect solution, or for those amines of very slight solubility, an equivalent of hydrochloric acid was added. To the resulting solution was added the equivalent amount of a saturated solution of the sulfonic acid (about 0.34 *N* at room temp.). Most of the salts precipitated shortly at room temperature; after filtration they were recrystallized from boiling water, or from aqueous alcohol if the water solubility was very small.

The solubility of the salts was determined by preparing a measured volume of a saturated water solution, chilling at 0° overnight, and sampling. The filtrates (or supernatant fluid) were evaporated in tared bottles over sulfuric acid; from the weights of the residues the water solubilities were calculated. The results are assembled in Table I.

Amino compounds which failed to give the desired sulfonate salts were *p*-bromoaniline, *o*-nitroaniline, 2,4-dinitroaniline, 2,4-dinitrophenylhydrazine, 1-amino-2-naphthol-4-sulfonic acid, diphenylamine and sulfanilic acid. This group of compounds is of extremely feeble basicity, and at the same time are quite insoluble in water and in dilute hydrochloric acid.

Another group of amines, most of which are soluble in water, failed to give precipitates when treated with the aqueous saturated sulfonic acid. This group includes: 2-amino-1-butanol, 2-amino-2-methyl-1,3-propanediol, bis-(hydroxyethyl)-*n*-butylamine, hydroxyethylbutylamine, hydroxyethyldi-*n*-butylamine, bis-(hydroxyethyl)-phenylamine, hydroxylamine (note the various hydroxylated amines) and in addition methyl and ethyl amines, dimethyl, diethyl and diisopropyl amines, morpholine, nicotine, nicotinic acid, pyridine and α- and β-picolines. Concentrated solutions of methyl- and ethylamines added to saturated solutions of the sulfonic acid gave the result of precipitation at −5°, but the precipitates proved to be largely the free sulfonic acid. In another trial the anhydrous vapors of ethyl- and methylamines were distilled directly into the saturated acid solution, but no precipitates were formed at room temperature.

It appears that the low molecular weight aliphatic amines form extremely soluble salts with dibenzofuran sulfonic acid which cannot be crystallized—the result being the hydrolysis of the salt to a sufficient degree to precipitate the acid component. This behavior contrasts sharply with that of ammonia which yields a quite insoluble salt, although it precipitates slowly. (It should be noted here that Mitchell and Bryant[8] encountered similar difficulties in preparing picrates of the lower aliphatic amines, several of which had to be added to a solution of picric acid in anhydrous ether before the salt could be recovered.

Notable is the easy precipitation of urea from dilute solutions. Of the compounds related to urea, thiourea precipitated very slowly, and was contaminated by I from which it

(1) Taken in part from the M.S. dissertations of Rode (1948) and Meintzer (1951).

(2) R. Wendland, C. H. Smith and R. Muraca, THIS JOURNAL, **71**, 1593 (1949).

(3) W. Borsche and R. Bothe, *Ber.*, **41**, 1941 (1908).

(4) H. Gilman, W. Bywater and P. Parker, THIS JOURNAL, **57**, 885 (1935).

(5) 3-Nitro-8-dibenzofuransulfonic acid had been formed by W. Borsche and B. Schacke, *Ber.*, **56**, 2501 (1923), but was isolated by them only as the sodium or potassium salt which is much less soluble.

(6) R. T. Wendland and C. H. Smith, *Proc. No. Dak. Acad. Science*, **III**, 31 (1949).

(7) F. W. Foreman, *Biochem. J.*, **14**, 451 (1920).

(8) J. Mitchell and W. M. D. Bryant, THIS JOURNAL, **65**, 123 (1943).