[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

Azo Dyes Derived from *o*-Hydroxybenzeneboronic Acid Anhydride

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Several new boron-containing azo dyes have been synthesized for testing in brain tumor therapy. Both ethylene chloride and nitrobenzene were found to be valuable recrystallizing solvents for this class of compounds. The infrared spectra of the compounds prepared show an absorption band at 9.1–9.2 μ which may be indicative of the carbon–boron bond.

Interest in boron-containing azo dyes[1,2] for use in the treatment of brain tumors has recently been stimulated by the work of Kruger.[3] This treatment involves the irradiation of cancerous tissue into which has been injected a compound containing the boron isotope 10.[4] Kruger[5] first proposed this type of therapy in 1940 and investigations along this same line have been carried out in the years following by a number of workers.[6–13] Borax was tested by Farr and co-workers,[12] and while this responded favorably to the irradiation treatment, it possessed several disadvantages, the most serious being the low differential uptake of the compound between normal and abnormal tissue,[13] the ratio being 1:2. This led to damage of a large amount of healthy tissue while the abnormal tissue was being destroyed.

Some non-boron containing dyes are absorbed preferentially by the cancerous tissue, and while many azo dyes are carcinogenic to the liver, their activity in this respect can be reduced, even to zero, by the introduction of certain groups such as the bromo, hydroxy, nitro and trifluoromethyl.[14] The azoboronic acids[1,2] which were tested by Kruger were also preferentially absorbed by the cancerous tissue, the differential uptake between the normal and abnormal tissue having a ratio of 1:100, thus indicating their superiority over borax in this respect. These encouraging results have demanded additional azoboron compounds as well as boron compounds in general for testing in the irradiation therapy of brain tumors.

In this most recent work, *o*-hydroxybenzeneboronic acid anhydride[15] was coupled with benzenediazonium chloride, *p*-bromobenzenediazonium chloride and the three nitrobenzenediazonium chloride isomers to give 2-hydroxy-5-(phenylazo)-benzeneboronic acid anhydride, 2-hydroxy-5-(*p*-bromophenylazo)-benzeneboronic acid anhydride and the three 2-hydroxy-5-(nitrophenylazo)-benzeneboronic acid anhydride isomers, respectively. Much lower yields of pure materials were obtained in these preparations than is usually experienced with dyes not containing the boronic acid or anhydride grouping. A 31% yield of pure 2-hydroxy-5-(phenylazo)-benzeneboronic acid anhydride was obtained while a 90% yield of its pure non-boron containing analog was prepared. In the case of the 2-hydroxy-5-(*p*-bromophenylazo)-benzeneboronic acid anhydride, a 20% yield of pure material was obtained while a 99% yield of the non-boron analog has been reported.[16] The three 2-hydroxy-5-(nitrophenylazo)-benzeneboronic acid anhydride isomers were all prepared in yields of less than 25%. Again the non-boron containing analogs can be prepared in much higher yields. For example, Elbs, *et al.*,[17] report a nearly quantitative yield of 4-(*o*-nitrophenylazo)-phenol. This much lower yield is partially explained by the deactivating effect of the boronic acid or anhydride group which certainly must be very pronounced in view of the electron deficiency of the boron atom. Decomposition during processing and purification perhaps also contribute to the lower yield.

Some improvement was noted in the yield and/or quality of the unpurified dye if the length of the coupling time was extended. Coupling times of 8 to 12 hr. were found to be noticeably superior to coupling times of 2 to 3 hr. For example, the melting point of the crude 2-hydroxy-5-(phenylazo)-benzeneboronic acid anhydride was increased 20 to 25° by use of this longer coupling time. There was no increase in the amount of crude material. The longer coupling time increased the amount of crude 2-hydroxy-5-(*p*-bromophenylazo)-benzeneboronic acid anhydride by 22% and raised the melting point by approximately 45°. Although this improvement probably carried through to the purified product, no such data were accumulated because of the difficulty involved in finding suitable purification procedures.

Recrystallization[18] of these dyes from an ethanol–water system, as was used by Snyder[1] for azoboronic acids derived from benzidine, failed to work satisfactorily. In most cases some purifica-

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(1) H. R. Snyder and Clay Weaver, THIS JOURNAL, **70**, 232 (1948).

(2) H. R. Snyder and S. L. Meisel, *ibid.*, **70**, 774 (1948).

(3) P. G. Kruger, *Radiation Research*, **3**, 1 (1955).

(4) Naturally occurring boron contains 18.83% of this isotope. See NBS Circular No. 499, p. 7.

(5) P. G. Kruger, *Proc. Natl. Acad. Sci.*, **26**, 181 (1940).

(6) P. A. Zahl, F. S. Cooper and J. R. Dunning, *ibid.*, **26**, 589 (1940).

(7) P. A. Zahl and F. S. Cooper, *Science*, **93**, 64 (1941).

(8) P. A. Zahl and L. L. Waters, *Proc. Soc. Exper. Biol. Med.*, **48**, 304 (1941).

(9) P. A. Zahl and F. S. Cooper, *Radiology*, **37**, 673 (1941).

(10) W. H. Sweet and M. Javid, *J. Neurosurg.*, **9**, 200 (1952).

(11) M. Javid, G. L. Brownell and W. H. Sweet, *J. Clin. Invest.*, **31**, 604 (1952).

(12) L. E. Farr, W. H. Sweet, J. S. Robertson, C. G. Foster, H. B. Locksley, D. L. Sutherland, M. L. Mendelsohn and E. E. Stickley, *Am. J. Roentgenol. Radium Therapy Nuclear Med.*, **71**, 279 (1954).

(13) L. E. Farr, J. S. Robertson and E. E. Stickley, *Proc. Nat. Acad. Sci.*, **40**, 1087 (1954); E. E. Stickley, *Am. J. Roentgenol. Radium Therapy Nuclear Med.*, **75**, 609 (1956).

(14) J. P. Greenstein, "Biochemistry of Cancer," 2nd Ed., Academic Press, Inc., New York, N. Y., 1954.

(15) H. Gilman, L. Santucci, D. R. Swayampati and R. O. Ranck, THIS JOURNAL, **79**, (1957), in press.

(16) F. D. Chattaway and H. R. Hill, *J. Chem. Soc.*, **121**, 2756 (1922).

(17) K. Elbs, O. Hirschel, F. Wagner, K. Himmler, W. Türk, A. Henrich and E. E. Lehmann, *J. prakt. Chem.*, **108**, 209 (1924).

(18) In all cases where purification was accomplished by recrystallization, the crystal form, if any, was rather indefinite. The form ranged from an apparently amorphous powder to an indefinite fibrous needle.

tion resulted as noted by an increase in the melting point over that of the crude. However, the lack of purity was well established by either the neutralization equivalent or quantitative boron analysis, or both, on the dyes in this series.

Dry ethylene chloride[19] was found to be a good recrystallizing solvent for the 2-hydroxy-5-(phenylazo)-benzeneboronic acid anhydride, the 2-hydroxy-5-(*p*-bromophenylazo)-benzeneboronic acid anhydride and the 2-hydroxy-5-(*o*-nitrophenylazo)-benzeneboronic acid anhydride. Even though none of these compounds was exceptionally soluble in the refluxing solvent, 1 g. to 1.5 g./liter, the material recrystallized readily and was far superior with respect to melting point and neutralization equivalent to material which could be obtained from any other solvent or solvent system including benzene, benzene–petroleum ether (b.p. 77–115°), acetone–water and chloroform–carbon tetrachloride.

Both the *m*- and *p*-isomers of 2-hydroxy-5-(nitrophenylazo)-benzeneboronic acid anhydride were purified successfully using nitrobenzene as the recrystallizing solvent. The high boiling point of this solvent makes it difficult to remove from the crystals, this being best accomplished by a washing with petroleum ether (b.p. 28–38°). This particular work was carried out before that done with ethylene chloride, and these particular compounds were never subjected to recrystallization from this solvent, although it is quite possible that the ethylene chloride would be quite adequate.

In studying the purification of these azoboronic acids, the possibility of using chromatographic techniques was investigated, most of the work being carried out on 2-hydroxy-5-(phenylazo)-benzeneboronic acid anhydride and a smaller amount on the 2-hydroxy-5-(*p*-bromophenylazo)-benzeneboronic acid anhydride, the 2-hydroxy-5-(*m*-nitrophenylazo)-benzeneboronic acid anhydride and the 2-hydroxy-5-(*p*-nitrophenylazo)-benzeneboronic acid anhydride. This method looked very promising in the early stages, but it was not possible to get material of a quality equal to that which could be obtained by recrystallization from either ethylene chloride or nitrobenzene. The best results were obtained using chloroform or a chloroform–carbon tetrachloride system as the solvent and eluent and a 1:2 mixture of Celite and silicic acid as the adsorbent.

Melting points have long been regarded as a criterion of purity for most organic compounds. However, this does not seem to hold true for azoboronic acids and in general for organoboronic acids.[15,20] Materials having the same neutralization equivalent may vary considerably in melting point. The rate of heating appears to be very important, much more so for this class of compounds than for the usual organic compound. Also, a wide difference in melting point is experienced depending on whether the sample is immersed initially in an unheated bath or in a previously heated bath. For example, partially purified 2-hydroxy-5-(*p*-bromophenylazo)-benzeneboronic acid anhydride had a melting point of 183–184° when the sample was placed in a previously heated bath and a melting point of 250–251° when the sample was immersed initially in the unheated bath.

In no instance was any effort made to prove the structure of the compounds other than from the information that could be obtained from the infrared spectra. This was believed to be adequate even though little is known about the exact position of the absorption bands for the azo group and the boronic acid anhydride substituent. The first of these possibly occurs at approximately 6.35–6.4 μ,[21,22] although none of the spectra obtained for these compounds showed any sharp absorption at this wave length. Many of the boronic acid spectra show an absorption band at 9.1–9.2 μ[20] as do these azoboronic acids. This may be indicative of the carbon–boron bond, although this has not been established definitely. The greatest use of the infrared was to determine positions of substitution on the aromatic rings as well as to prove the existence of the hydroxy, nitro and bromo groups whose absorption bands are well known. Actually, the method of preparation should establish the structure reasonably well, and the color should give some evidence for the existence of the azo group while the neutralization equivalent should verify the presence of a boronic acid or anhydride group.

Additional attempts were made to produce other boron-azo compounds, but it has not yet been possible to purify these to an acceptable degree. These compounds include those formed by coupling *o*-hydroxybenzeneboronic acid anhydride with diazotized sulfanilic and *p*-aminobenzoic acids and tetrazotized benzidine.

Experimental[23]

2-Hydroxy-5-(phenylazo)-benzeneboronic Acid Anhydride.—The diazotization of 18.6 g. (0.2 mole) of aniline was carried out according to German Dyestuff information.[24] The benzenediazonium chloride solution was then added over a period of 30 minutes to a stirred solution composed of 22 g. (0.55 mole) of sodium hydroxide, 24 g. (0.2 mole) of 2-hydroxybenzeneboronic acid anhydride and 200 ml. of water, cooled to 5° by means of an ice–salt-bath; 200 g. of ice was added to the reaction flask immediately before the addition was started. Agitation was continued for 11 hr. at 0–5° following completion of the addition. The reaction mass was then filtered, the filter cake was suspended in 2000 ml. of water at 40° and was acidified by the addition of 10% hydrochloric acid. After cooling to room temperature, the precipitated solid was removed by filtration and was dried in a vacuum oven at 45°; 45 g. (100%) of crude reddish-brown material having a melting point range of 178–188° was obtained. Acidification of the filtrate from the reaction mass gave less than 1 g. of additional crude material which was combined with the bulk of the crude.

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[19] Eastman Kodak white label 1,2-dichloroethane distilled over phosphorus pentoxide was used for this purpose.

[20] L. Santucci and H. Gilman, THIS JOURNAL, **79**, in press (1957).

[21] L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1954, p. 229.

[22] H. M. Randall, R. G. Fowler, N. Fuson and J. R. Dangl, "Infrared Determination of Organic Structures," D. Van Nostrand Co., Inc., New York, N. Y., 1949, p. 52.

[23] All melting points are uncorrected. In taking the melting points, the sample generally was immersed in a previously unheated bath. However, when it was noted that the rate of heating affected the melting point appreciably, a previously heated bath was used. The neutralization equivalents were obtained by complexing the compound with D-mannitol in an ethanol–water system and titrating with standard sodium hydroxide using a Beckman model G *p*H meter to detect the end-point.

[24] I. G. Farbenindustrie, British Intelligence Objectives Subcommittee, Report No. 1548, p. 65 (PB85593).

The crude material was washed with approximately 500 ml. of dry ethylene chloride. This removed a large portion of the impurities, the washed material now having a melting point range of 210–220°. This material was recrystallized three times from ethylene chloride to give 14 g. (31%) of bright yellow fibrous material melting at 236–238°. After each recrystallization the volume of the filtrate was reduced in order to recover the maximum amount of material. The infrared spectrum supported the expected structure.

Anal. Calcd. for $C_{12}H_9BN_2O_2$: B, 4.84; N, 12.51; neut. equiv., 224. Found: B, 5.08, 4.96; N, 12.63, 12.42; neut. equiv., 226, 227.

In an experiment where a 3-hr. coupling time was employed, the crude yield was 100% and the material had a melting point range of 150–185°. A 9-hr. coupling time gave a 94% yield of material isolated in two portions; the first had a melting point range of 170–180° and represents 21.3% of the yield while the second portion had a melting point range of 188–192° and represents 72.7% of the yield.

Chromatographic purification of this dye was studied to a reasonable extent without any outstanding success being attained. In one experiment, 2.25 g. of crude dye (m.p range 188–197°) was dissolved in 500 ml. of chloroform and was chromatographed on a column composed of 225 g. of a 1:2 mixture of Celite and silicic acid. The column was eluted with additional chloroform. Nine fractions were collected. Fractions 1 through 3 were semi-solids and represented 20% of the original weight. No melting point was obtained on this portion of the material. Fraction 4 which represented 11% of the original weight had a melting point range of 140–150°. Fractions 5 through 8 all had melting points higher than the crude, fraction 6 having the maximum range of 204–209°. This series represents 49% of the original weight. Fraction 9 was again a semi-solid, no melting point being obtained, and represents 4.5% of the original weight. Fraction 6 had a neutralization equivalent of 234 which indicates material of fair purity. The calculated value for the neutralization equivalent for the anhydride is 224.

The use of a 1:1 mixture of chloroform and carbon tetrachloride solution as the solvent and eluent was superior in one respect to chloroform alone in that sharper separations could be made but was inferior in another in that the solubility of the compound was less.

A 1:1 mixture of Celite and silicic acid showed no advantage while activated alumina was of no value whatsoever.

2-Hydroxy-5-(*p*-bromophenylazo)-benzeneboronic Acid Anhydride.—Eight and six-tenths grams (0.05 mole) of *p*-bromoaniline was diazotized according to the method of Gomberg and Bachmann.[25] This *p*-bromobenzenediazonium chloride solution was then added over a 10-minute period to a stirred solution composed of 5.5 g. (0.135 mole) of sodium hydroxide, 6.0 g. (0.05 mole) of 2-hydroxybenzeneboronic acid anhydride, 50 ml. of water and 50 g. of ice; and cooled in addition with an ice–salt-bath. Agitation was continued for 9 hr. following completion of the addition, the temperature being maintained at 0–5°. The reaction mixture was filtered, the filter cake was suspended in 500 ml. of water at 40° and was then acidified by the addition of 10% hydrochloric acid. The precipitated solid was removed by filtration after cooling to 20° and was dried at 45° in the vacuum oven. The dried material weighed 14.3 g., had a melting point range of 170–190° and represents a crude yield of 89%. Acidification of the filtrate from the reaction mass gave a small amount of additional, but worthless, material.

The brown crude material was washed with 200 ml. of dry ethylene chloride, a process which removed a large portion of the impurities, and the washed material was recrystallized three times from ethylene chloride to give 3 g. of bright yellow and rather fibrous material melting at 350–355°. This weight represents a 20% yield of pure material. After each recrystallization, the volume of the solvent was reduced in order to recover the maximum amount of material. The infrared spectrum supported the expected structure.

Anal. Calcd. for $C_{12}H_8BBrN_2O_2$: B, 3.57; Br, 26.38; neut. equiv., 302.9. Found: B, 3.95, 3.70; Br, 25.65, 25.44; neut. equiv., 308.

In an experiment where a 30-minute coupling time was employed, an 86% yield of crude material having a melting point range of 130–141° was obtained.

In the attempted chromatographic purification of this compound, 12 g. of crude (m.p. range 180–200°) was partially dissolved in 500 ml. of benzene and was poured on a column containing 450 g. of a 1:2 mixture of Celite and silicic acid. The purest material which could be obtained on elution with additional benzene had a melting point range of 210–220° which is far below that of the recrystallized material. Poorer separations were obtained when chloroform was substituted for benzene as the solvent and eluent. A mixture of chloroform and carbon tetrachloride (1:1) was superior to chloroform alone.

2-Hydroxy-5-(*o*-nitrophenylazo)-benzeneboronic Acid Anhydride.—Two and eight-tenths grams (0.02 mole) of *o*-nitroaniline was diazotized according to the method employed by Smith and Boyer.[26] The filtered *o*-nitrobenzenediazonium chloride solution was added to a stirred solution composed of 2.76 g. (0.023 mole) of 2-hydroxybenzeneboronic acid anhydride, 2.2 g. (0.055 mole) of sodium hydroxide and 25 ml. of water cooled to 0–5° by means of an ice–salt-bath. Agitation was continued for 2 hr. following completion of the addition, the temperature being maintained below 5°. The reaction mass was filtered after which the filter cake was suspended in 200 ml. of water and acidified by the addition of 10% hydrochloric acid. The product was filtered, washed and air-dried to give 4 g. of a brown product having a melting point range of 137–198°. This weight represents a 75% crude yield. Acidification of the filtrate from the reaction mixture gave a very small amount of even poorer quality material.

The crude material was recrystallized two times from benzene to give 1.3 g. of an orange colored solid melting at 213–214°. This weight represents a 24% yield of reasonably pure material. The infrared spectrum supported the structure.

Anal. Calcd. for $C_{12}H_8BN_3O_4$: N, 15.61; neut. equiv., 269. Found: N, 15.18, 15.24; neut. equiv., 290.[27]

After it was found that ethylene chloride was a good recrystallizing solvent for this type of compound, some of the benzene-recrystallized material, obtained in the experiment above, was also recrystallized from ethylene chloride. This gave orange colored material having a melting point of 236–238° and a neutralization equivalent closer to the anhydride than the material which was recrystallized from benzene.

Anal. Calcd. for $C_{12}H_8BN_3O_4$: B, 4.02; N, 15.61; neut. equiv., 269. Found: B, 4.46, 4.37; N, 15.15, 15.43; neut. equiv., 280.

In another experiment where an 8-hr. coupling time was used, a 78% yield of material having a melting point range of 150–180° was obtained. Recrystallization of this from ethylene chloride gave a 24% yield of material melting over the range of 230–235°. No chromatographic purification was attempted on this compound.

2-Hydroxy-5-(*m*-nitrophenylazo)-benzeneboronic Acid Anhydride.—To a stirred solution of 6.00 g. (0.05 mole) of *o*-hydroxybenzeneboronic acid anhydride and 10.0 g. (0.25 mole) of sodium hydroxide in 50 ml. of water, cooled to 0–5° in an ice–salt-bath, was added slowly a solution containing 6.9 g. (0.05 mole) of *m*-nitroaniline diazotized according to the method of Kaslow and Summers.[28] The alkaline reaction mixture (*p*H approximately 8) was stirred for 10 hr. at 0–5° and was then filtered. The orange filter cake was suspended in 500 ml. of water and was acidified by the addition of 10% hydrochloric acid. A 93% (12.37 g.) yield of material melting over the range of 168–180° was obtained. Acidification of the filtrate from the reaction mixture gave no appreciable amount of additional material.

The crude product was recrystallized twice from nitrobenzene, a Norit-A treatment being employed and care being taken not to heat the solution above 95°. Thorough washing of the recrystallized material with petroleum ether (b.p.

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(25) M. Gomberg and W. E. Bachmann, *Org. Syntheses*, **8**, 42 (1928).

(26) P. A. S. Smith and J. H. Boyer, *ibid.*, **31**, 14 (1951).

(27) The calculated values shown in this analysis are for the anhydride. At the time this material was prepared it was believed that the acid was being formed, but later work on boron-azo compounds of this type indicated that it was more likely the anhydride. The calculated values for the acid are: N, 14.64; neut. equiv., 287.

(28) C. E. Kaslow and R. M. Summers, *Org. Syntheses*, **33**, 56 (1953).

28–38°) was done in order to remove the nitrobenzene completely. A 20.5% (2.74 g.) yield of yellow material melting at 220–222° was obtained. The analytical sample was recrystallized a third time from nitrobenzene to give material melting at 221–222°. The infrared spectrum supported the expected structure.

Anal. Calcd. for $C_{12}H_8BN_3O_4$: C, 53.60; H, 3.00; N, 15.61; B, 4.02; neut. equiv., 269.06. Found: C, 53.73, 53.80; H, 3.27, 3.18; N, 15.39, 15.41; B, 4.00, 3.98; neut. equiv., 285.7.

In another experiment where the reaction mass was stirred at 0–5° for 8 hr., an 82.5% yield of crude material melting over the range of 144–170° was obtained. This gave a 17.5% yield of pure material.

For best results, the optimum *p*H was found to be about 8. Both sodium bicarbonate and sodium acetate were used as buffers in some experiments, but they did not seem to be particularly useful. Chromatographic purification was tried without any great success.

2-Hydroxy-5-(*p*-nitrophenylazo)-benzeneboronic Acid Anhydride.—To a stirred solution of 6.00 g. (0.05 mole) of *o*-hydroxybenzeneboronic acid anhydride and 10 g. (0.25 mole) of sodium hydroxide in 50 ml. of water, cooled in an ice–salt-bath to 0–5°, was added slowly a solution of 6.9 g. (0.05 mole) of *p*-nitroaniline diazotized according to the method of Ropp and Coyner.[29] The alkaline reaction mixture (*p*H approximately 8) was stirred at −10 to 0° for 6 hr. and was then filtered. The brick-red filter cake was suspended in 500 ml. of water, and this was acidified by the addition of 10% hydrochloric acid. A 70% (9.35 g.) yield of material melting over the range of 184–200° was obtained. Acidification of the filtrate from the reaction mixture gave an additional 0.51 g. (3.8%) of material having a melting point range of 205–225°. The combined crude materials could not be recrystallized directly from any solvent with much success. The crude was therefore extracted with a small amount of hot acetone and the residue was dissolved in 250 ml. of hot acetone. The insoluble portion was removed by filtration and the dye was reprecipitated from the filtrate by the addition of 900 ml. of warm water. The material, now having a melting point of 207–208°, was recrystallized from nitrobenzene using the procedure described above for the *m*-isomer; 1.4 g. (10.4%) of orange product melting sharply at 241.8–242° was obtained. The analytical sample was recrystallized one additional time from nitrobenzene to give material melting at 242–242.2°. The infrared spectrum supported the expected structure.

Anal. Calcd. for $C_{12}H_8BN_3O_4$: N, 15.61; B, 4.02; neut. equiv., 269.06. Found: N, 15.39, 15.16; B, 3.92, 4.22; neut. equiv., 290.1.

In another experiment an 84% yield of crude material was obtained when an 8-hr. coupling time at 0–5° was used. When the coupling time was increased to 10.5 hr., the amount of crude material did not change, but the product melted at 187° instead of over the range reported above. In this particular experiment 17% of the original *o*-hydroxybenzeneboronic acid anhydride was recovered. The pure yield was not determined exactly in these last two cases. The use of sodium bicarbonate as a buffer did not show any advantages. All of the couplings were run at a *p*H of 8. Chromatographic purification also was attempted on this compound, but it did not work very satisfactorily.

Acknowledgment.—We wish to thank Mr. Robert McCord and Mr. E. Miller Layton of the Ames Laboratory of the Atomic Energy Commission for the infrared spectra. We also wish to acknowledge the support of the Division of Biology and Medicine of the United States Atomic Energy Commission who have made this work possible. The results of the biological testing of these compounds will be reported by Dr. Otho D. Easterday of the Brookhaven National Laboratory.

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[29] G. A. Ropp and E. C. Coyner, *Org. Syntheses*, **31**, 80 (1951).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE STATE UNIVERSITY]

Terpenoids. XXVII.[1] The Structure of the Cactus Triterpene Myrtillogenic Acid[2]

BY CARL DJERASSI AND H. G. MONSIMER[3]

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Myrtillogenic acid has been shown to be 3β,16β,28-trihydroxy-Δ^{12}-oleanen-29-oic acid (Ia) by various degradation reactions and by conversion to longispinogenin triacetate (XIIb).

During an investigation[4] of the triterpene composition of the genus *Myrtillocactus*, there was isolated from several species in small amounts a new triterpene acid. Since this substance has not been encountered in any other genus of the cactus family, we have named it "myrtillogenic acid" and the present paper is concerned with its structure elucidation.

Myrtillogenic acid was separated from the other triterpenes as the methyl ester ($C_{31}H_{50}O_5$) and then saponified to the free acid ($C_{30}H_{48}O_5$). Mild acetylation with acetic anhydride–pyridine of either the ester or the acid led to the corresponding triacetate, thus accounting for all oxygen functions. The ease of acetylation suggested that the three hydroxyl groups were either equatorially oriented or that one or more were primary. Membership in the β-amyrin class of triterpenes was indicated[5] by the course of the selenium dioxide oxidation of triacetyl methyl myrtillogenate which furnished a $\Delta^{11,13(18)}$-diene (subsequently shown to be II) with the typical[6] triple ultraviolet absorption maxima at 240, 248 and 258 mμ.

All of the triterpene acids which have been encountered[1] so far among the *Cactaceae* contained the carboxyl group at C-17, but two different lines of evidence could be presented that this was not the case with myrtillogenic acid. Oleanolic acid (IIIa)

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[1] Paper XXVI, "Cactus Triterpenes," by C. Djerassi in "Festschrift Arthur Stoll," Birkhäuser, Basel, 1957, pp. 330–352.

[2] This investigation was supported by a research grant (No. RG-3863) from the Division of Research Grants of the National Institutes of Health, U. S. Public Health Service.

[3] Taken from part of the Ph.D. dissertation of H. G. Monsimer.

[4] C. Djerassi, S. Burstein, H. Estrada, A. J. Lemin, A. E. Lippman, A. Manjarrez and H. G. Monsimer, THIS JOURNAL, in press.

[5] That this is not an unambiguous criterion, as had been believed earlier, was demonstrated recently in the case of the cactus triterpene dumortierigenin (C. Djerassi, C. H. Robinson and D. B. Thomas, *ibid.*, **78**, 5685 (1956)) which belongs to the β-amyrin series but does not react to any appreciable extent with selenium dioxide. However, a positive reaction, as was observed with myrtillogenic acid, appears to be conclusive.

[6] *Cf.* L. Ruzicka, G. Müller and H. Schellenberg, *Helv. Chim. Acta*, **22**, 767 (1939); D. H. R. Barton and C. J. W. Brooks, *J. Chem. Soc.*, 257 (1951).