D(+)-*Apiose from the Monocotyledon,* Posidonia australis.

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(*With a Note on the Stereochemistry and Nomenclature.* By R. S. Cahn.)

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D(+)-Apiose has been identified as the main monosaccharide liberated, on mild acid hydrolysis, from both leaves and naturally "retted" residual fibres ("marine fibre") of *Posidonia australis* (Potamogetonaceae). This sugar has hitherto been known only as a component of the flavone glycosides derived from parsley (*Apium petrosilinum*, Umbelliferae). Two useful crystalline compounds of D-apiose are described, the di-*O-iso*propylidene derivative and the 2 : 5-dichlorophenylosazone. The latter contains no asymmetric centre and could be obtained from either the D- or the L-form of the sugar.

The stereochemistry and nomenclature of cyclic apiose derivatives are discussed.

As a sequel to the re-examination of the nature of the cellulose of the leaves and "residual fibre" of *Posidonia australis* by Bell (*J.*, 1952, 3649) unsuccessful attempts were made to isolate a xylan from the leaves of this plant. Dr. J. C. Earl, who kindly made his original notes available to us, had previously attempted, without success, to identify D-xylose among the products formed by treatment of the leaves and fibres with hot 2% sulphuric acid; neither the phenylosazone nor an identifiable oxidation product of D-xylose could be isolated.

We subjected both dried fresh leaves and residual fibres to treatment with hot 2% (w/v) sulphuric acid and examined the hydrolysate by paper chromatography, using aniline phthalate to reveal the sugar spots. Xylose, arabinose, and two bright yellow spots, in positions corresponding respectively to glucose and rhamnose, were observed.

It was therefore decided, as a preliminary to assaying the xylose in the hydrolysate, to attempt positive identification of the monosaccharides through their *O-iso*propylidene derivatives (Bell, *J.*, 1947, 1461). The product, obtained by treatment of the dried mixture of sugars with acetone containing 5% (v/v) of sulphuric acid, consisted largely of crystals unexpectedly insoluble in cold water. After recrystallisation from hot water, these crystals analysed exactly for a di-*O-iso*propylidenepentose but melted at 82°, being thus distinguished from similar derivatives of any of the "normal" pentoses. No unsubstituted hydroxyl groups could be detected.

When hydrolysed by N-acid this substance yielded a syrup which behaved chromatographically exactly as the "rhamnose" spot mentioned above. It did not, however, yield acetaldehyde on oxidation by periodate, nor could evidence be obtained by Edward and Waldron's procedure (*J.*, 1952, 3631) that the sugar contained a "secondary" deoxy-grouping (*i.e.*, $-CH_2-$).

Paper-chromatographic investigation under the exactly controlled conditions of Isherwood and Jermyn (*Biochem. J.*, 1951, **48**, 515) showed that the sugar was not an unbranched 2-hexulose, a hexose, a straight-chain pentose, or a 6-deoxyhexose. With ethyl acetate–pyridine–water (2 : 1 : 2) as solvent the R_F value was 0·40 while that of rhamnose was 0·38. The other 6-deoxyhexoses, in this solvent, have very different R_F values.

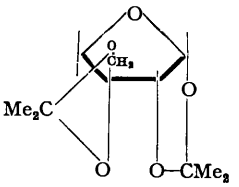
Analytically, although it did not yield furfuraldehyde on acid treatment, the sugar appeared to be without doubt a pentose and, from its high R_F value, probably existed in solution as a furanose. The ease of formation of a di-*O*-*iso*propylidene derivative suggested that examination of a paper chromatogram in presence of boric acid might provide a clue to its structure since boric acid is known to form complexes with vicinal hydroxyl groups attached to a five-membered ring (Böeseken, *Adv. Carbohydrate Chem.*, 1949, **4**, 189; Barker and Smith, *Chem. and Ind.*, 1954, 19). The R_F values given in the annexed Table show that the R_F of the unidentified sugar is markedly affected by boric acid. The only well-known pentose, not examined by Isherwood and Jermyn (*loc. cit.*), which might behave in this way, is apiose (cf. Hudson, *Adv. Carbohydrate Chem.*, 1949, **4**, 57), a sugar possessing a branched chain. The sugar from *Posidonia* and authentic D-apiose (prepared from apiin) showed identical behaviour on the paper chromatogram.

R_F values in *n*-butanol–water.

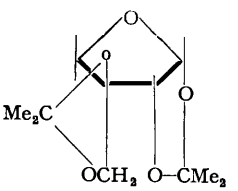
	Glucose	Rhamnose	Fucose	Galactose	*P. australis* sugar
Without boric acid ...	0·08	0·23	0·16	0·08	0·26
With boric acid	0·06	0·24	0·16	0·07	0·04

Proof that the sugar is in fact D(+)-apiose was afforded by the preparation from an authentic sample, isolated by controlled hydrolysis of apiin, of the di-*O*-*iso*propylidene derivative and the 2 : 5-dichlorophenylosazone. These were identical with the derivatives prepared from the *Posidonia* sugar, as were samples of the previously known *p*-bromophenylosazone (Vongerichten and Müller, *Ber.*, 1906, **39**, 235). It should be noted that osazones of apiose have lost their asymmetric centre and do not therefore distinguish between D- and L-isomers.

The structure of the di-*O*-*iso*propylidene derivative cannot yet be definitely stated. Arguing from the tendency of the *iso*propylidene radical to engage vicinal *cis*-hydroxyl groups, especially when one is a " reducing " hydroxyl group, it seems logical to assign positions 1 and 2 to one substituent. This would leave the remaining hydroxyl groups on positions 3 and 5 (which are not vicinal) to bear the second *iso*propylidene group. As discussed below, ring-formation in apiose results in the production of a new asymmetric carbon atom at position 3. At present we have no means of learning the configuration of this new asymmetric centre. We therefore suggest that the apiose derivative in question be for the present designated " probably 1 : 2-3 : 5-di-*O*-*iso*propylidene-D-apio-(D or L)-furanose," *i.e.* (*A*) or (*B*).



(*A*)



(*B*)

The form in which the apiose radicals are present in *Posidonia* is under investigation; it seems clear, since the sugar is liberated from the plant tissues only after acidic treatment, that they must be glycosidically bound. Hitherto this sugar has been known only in the form of the disaccharide-flavone glycoside apiin isolable from parsley (Hemming and Ollis, *Chem. and Ind.*, 1953, 85; cf. Hudson, *loc. cit.*). Our observations that, chromatographically, the sugar may be mistaken for rhamnose, and that it is present in a plant of a species totally different from an umbellifer, suggest that it may be more widely distributed than has hitherto been suspected. *Posidonia australis* does not appear to contain apiin.

Nothing is known of the biological behaviour of this sugar; we are unaware of any literature referring to tests done with micro-organisms. It is noteworthy that an adenoside of (+)-3-deoxyapiose (cordycepose) isolated by Bentley, Cunningham, and Spring (*J.*, 1951, 2299, 2301) has some antibiotic properties and that other branched monosaccharides occur in certain established antibiotics, *e.g.*, streptose in streptomycin (Lemieux and Wolfrom, *Adv. Carbohydrate Chem.*, 1948, **3**, 337) and (—)-mycarose in magnamycin (Regna, Hochstein, Wagner, and Woodward, *J. Amer. Chem. Soc.*, 1953, **75**, 4625). It is suggested, with all reserve, that the resistance to natural decomposition processes, so marked in the fibres of *Posidonia australis* and its close relative, *Zostera marina*, may be due to the presence of some derivative of D-apiose (cf. Winterbottom, South Australia Dept. Chemistry, 1917, Bull. No. 4, p. 28).

Stereochemistry and Nomenclature.*

The furanose forms of apiose present novel features of asymmetry and nomenclature.

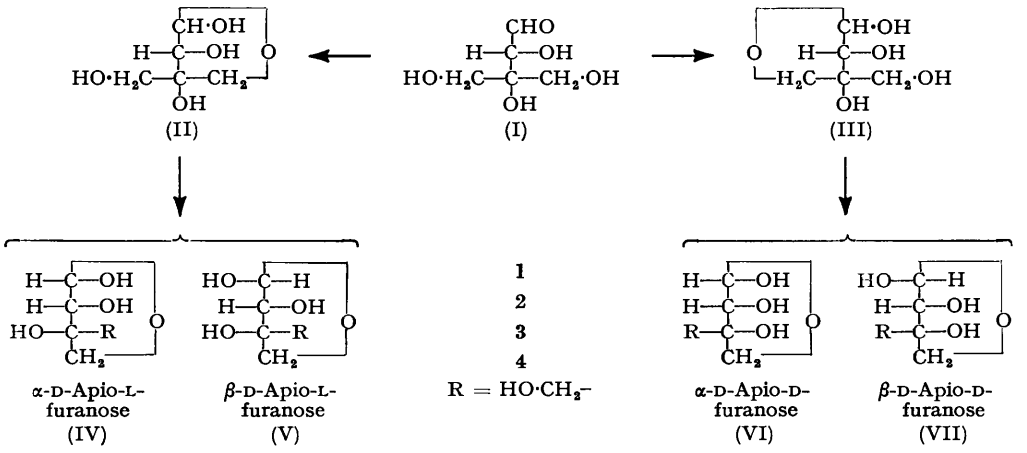
The *aldehydo*-form (I) of apiose may be called D-apiose (or, more specifically *aldehydo*-D-apiose), the prefix D being derived directly from the glyceraldehyde convention and the customary carbohydrate convention.

When furanose-ring formation takes place, as in (II) or (III), two further centres of asymmetry are created: one of these, that at position 1, is covered by the customary αβ nomenclature for glycosidic anomers; the second, that at position 3, is not covered by the normal rules of carbohydrate nomenclature (*J.*, 1952, 5108).

It will be wise always to write apiose projection formulae in the conventional way, with the primary alcoholic group which is involved in ring formation at the bottom (if the oxygen bridge starts vertically from position 1, it is immaterial whether the oxygen atom is written to the left or to the right since, in so far as the projection formulæ have pictorial meaning, the ring lies behind the plane of the paper). Thus the formula (II) should be re-written as (IV) and (V), and the formula (III) as (VI) and (VII).

Numbering should be 1 to 4, from the glucosidic position to the other end of the furanose ring, with the free CH_2·OH group numbered 5.

According to the direction of ring-closure, D-apiose will give one or more of the four isomers (IV)—(VII). L-Apiose similarly gives the mirror images of these, a total of eight



optically active individuals, as required for a compound containing three different asymmetric centres.

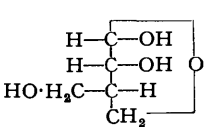
According to the customary nomenclature of carbohydrates (*ibid.*, rule 5) the con-

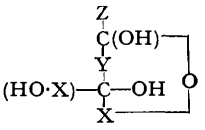
* The Chemical Society's Carbohydrate Nomenclature Sub-Committee has not yet considered branched-chain sugars. The appearance of the Editor's name as an author must not be construed as committing the Society to the nomenclature proposed in this paper.

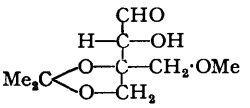
figurational prefix D or L follows the asymmetry of the highest-numbered asymmetric atom. But the *aldehydo*-form (I) of D-apiose then generates two D-furanose forms (VI and VII) and two L-furanose forms (IV and V); similarly, *aldehydo*-L-apiose generates two L- and two D-furanose forms. The generic relations of the individuals would thus be obscured. For example, by classical principles, (II; = III + IV) might be termed αβ-L-*threo*apiose, whilst (III; = VI + VII) might be termed αβ-D-*erythro*apiose, yet both are derived from *aldehydo*-D-apiose (I).

We therefore suggest a new form of nomenclature for this sugar. Let " D-apio " refer always to the asymmetry of the open-chain form (I), *i.e.*, to position 2. When this form cyclises, " furanose " should be added to the stem, as customary, but now together with a second D defining the stereochemistry at position 3, giving D-apio-D-furanose, etc. Addition of the customary prefixes α, β completes the names, which then are as under the formulæ (IV)—(VII). In these designations the three stereochemical prefixes denote, in order, the configuration at positions 1, 2, and 3. The mirror image of a particular isomer is that in which all three stereochemical prefixes are simultaneously reversed.

Bentley, Cunningham, and Spring (*loc. cit.*) encountered a similar case with cordycepose, a 3-deoxyapiose (*e.g.*, VIII), but in their work did not need a precise stereochemical nomenclature. Since there is no hydroxyl group at position 3 there is no firm convention or rule by which to assign a prefix *threo* or *erythro*, or a symbol D or L, to the name cordycepose in connexion with the stereochemistry of its furanose forms. Thus it is preferable to designate the furanose forms of cordycepose in terms of 3-deoxyapiose; the nomenclature suggested above is adequate also for the 3-deoxy-derivative.


(VIII)


(IX)


(X)

The specific case of apiose can be generalised by the expression (IX), the essential features calling for the revised nomenclature being that ring closure creates new asymmetry at a carbon atom which bears a higher number than that of any other asymmetric atom in the chain. Apiose is (IX) in which X = CH_2, Y = CH·OH, and Z = H, and the nomenclature proposed will deal adequately with some analogous cases; but others can be conceived, *e.g.*, (X), in which other considerations may ensue. Accordingly the nomenclature proposed for apiose (and cordycepose) should be regarded as an interim step until more branched-chain sugars presenting this particular problem have been isolated; it will then be clearer whether the classical carbohydrate conventions can be adequately modified to meet practical demands, or whether recourse must be had to one of the general schemes (cf. Cahn and Ingold, *J.*, 1951, 612; Klyne, *Chem. and Ind.*, 1951, 1022; McCasland, cited by Patterson, *Chem. Eng. News*, 1954, **32**, 434).

Experimental

Evaporations were done under reduced pressure below 40°.

Hot Acid Extraction of Posidonia *Leaves.*—To 100 g. of air-dried *Posidonia* leaves (water content 10%) were added N-sulphuric acid and water in proportions such that the final acid concentration (by titration) was ~0·4N. The total volume of liquid was 850 ml. The whole was then heated at 100° for 1 hr., cooled, and filtered; the residue was well pressed and washed twice with water. After neutralisation by barium carbonate, the filtered solution and washings were shaken with charcoal and again filtered, through Celite and charcoal. The filtrate, acidified with acetic acid, was evaporated. (This preliminary acidification was found to be essential to prevent aldose–ketose interconversion.)

Samples of " residual fibres " were treated in the same way.

Paper-chromatographic Examination of the Hydrolysate.—Since the neutralised hydrolysates contained inorganic salts which interfered with direct chromatographic examination numerous trials were done before a satisfactory procedure was found. (It was early noted that D-apiose

is remarkably sensitive to alkali; traces of soluble alkali in barium carbonate preparations cause the subsequent production of two spots on the chromatogram, presumably due to aldose–ketose transformation.) In view of the observations of Hulme (*Nature*, 1953, **171**, 610) and of Rebenfeld and Pacsu (*J. Amer. Chem. Soc.*, 1953, **75**, 4371) regarding the destructive and isomerising effects of strongly basic resins, it was considered advisable not to attempt deionisation by such means. Direct chromatography of the acid hydrolysate by Gaillard's method (*Nature*, 1953, **171**, 1160) was found to give very satisfactory results. Using as solvent benzene–butanol–pyridine–water (1 : 5 : 3 : 3), developing for 16 hr. at room temperature, and spraying with aniline hydrogen phthalate (Partridge, *Nature*, 1948, **164**, 443) gave the results tabulated on p. 3703 for material from both leaves and fibres.

Isolation of the Di-O-iso*propylidene Derivative of* D-*Apiose* (cf. Bell, 1947, *loc. cit.*).—The mixture of sugars, obtained by 0·4N-acid treatment, was dissolved in water (50 ml.) and evaporated in presence of Celite (4 g.). The resulting mass, after drying at 0·05 mm. over phosphoric oxide, was agitated with dry acetone (300 ml.) containing 5% (v/v) of sulphuric acid for 6 hr. at room temperature. The final product was largely crystalline. Recrystallised from water containing a trace of ammonia, needles or plates were found with m. p. 81—83° and $[\alpha]_D^{19}$ +56·4° (*c*, 3·2 in EtOH) (Found : C, 57·5; H, 7·7. $C_{11}H_{18}O_5$ requires C, 57·4; H, 7·9%). In attempts to acetylate this material it was recovered unchanged. Yields were : from leaves, 2·6% (rather less from a second batch); from fibre, 2·5%; from water-extracted leaves, <0·1%.

Authentic D(+)-apiose was treated as above. The product had m. p. and mixed m. p. 81—83°, $[\alpha]_D^{19}$ +55·6° (*c*, 1·35 in EtOH) (Found : C, 57·5; H, 7·7%).

Apiose 2 : 5-*Dichlorophenylosazone.*—To the sugar (75 mg.) obtained after acid hydrolysis of the di-*O-iso*propylidene derivative (obtained from leaves), were added water (1 ml.), 2 : 5-dichlorophenylhydrazine (300 mg.), ethanol (1 ml.), 8N-acetic acid (2 ml.), and sufficient sodium acetate to give a final pH of 4·5. The mixture was heated (~100°) for 45 min. A yellow solid separated; as this was considered possibly to be a hydrazone it was dissolved by dropwise addition of ethanol. After heating of the solution for a further 5 min. and cooling, the yellow product was collected and recrystallised from ethanol to which water was added dropwise.

Apiose 2 : 5-*dichlorophenylosazone* (needles) had m. p. 188·5—190·5°, not depressed on admixture with material prepared from authentic D-apiose (Found : C, 44·5; H, 3·4; N, 12·1; Cl, 30·3. $C_{17}H_{16}O_3N_4Cl_4$ requires C, 43·8; H, 3·4; N, 12·0; Cl, 30·5%). Authentic apiose 2 : 5-dichlorophenylosazone had m. p. 187—188° (Found : C, 44·3; H, 3·8; N, 13·1; Cl, 32·3%).

Apiose p-*Bromophenylosazone.*—This was prepared essentially as described for the dichloro-derivative. After heating of the reaction mixture for 1·5 hr., the resulting crystals, recrystallised from aqueous alcohol, had m. p. 209·5—210·5° (corr.). Vongerichten and Müller (*loc. cit.*) give m. p. 211—212°. Yields of both ozazones were very satisfactory.

As regards one of us (F. A. I.) the work described in this paper was carried out as part of the programme of the Food Investigation Organisation of the Department of Scientific and Industrial Research; Mr. F. C. Barrett performed much of the chromatographic work described. We are grateful to Dr. H. B. S. Womersley of the Botany Department, Adelaide University, for generous supplies of *Posidonia* leaves and fibres. One of us (N. E. H.) is indebted to the University of Western Australia for a Hackett Studentship and to Girton College, Cambridge, for an Ethel Sargant Studentship.

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