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BENZISOXAZOLE AND BENZISOTHIAZOLE ANALOGS OF AUXIN

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Abstract—In order to ascertain to what extent benzisoxazole and benzisothiazole can substitute the indole nucleus in auxin, several benzisoxazole and benzisothiazole analogs of indole-3-acetic acid were synthesized and their activity as plant hormones tested. The results of the slit-pea-stem curvature test are reported; among other compounds, 5-chloro-1, 2-benzisothiazole-3-acetic acid was the most active, showing about 330 per cent of the activity of indole-3-acetic acid.

THE SYNTHESIS of 1,2-benzisoxazole-3-acetic acid from the reaction between 4-hydroxycoumarin and hydroxylamine has been dealt with in a previous note.[1] The compound, an isoster of the naturally-occurring plant regulator indole-3-acetic acid, showed an interesting auxin-like activity. Since it seemed interesting to establish whether benzisoxazole and benzisothiazole could substitute the indole nucleus in auxin, we decided to test more benzisoxazole and benzisothiazole analogs.

We wish to report now the results of these tests as well as the synthesis of several 1,2-benzisoxazole- and 1,2-benzisothiazole-3-acetic acids, through an extension of the reaction of 4-hydroxycoumarin with hydroxylamine to substituted 4-hydroxycoumarins and 4-hydroxy-1-thiocoumarins, according to the scheme shown in Fig. 1.

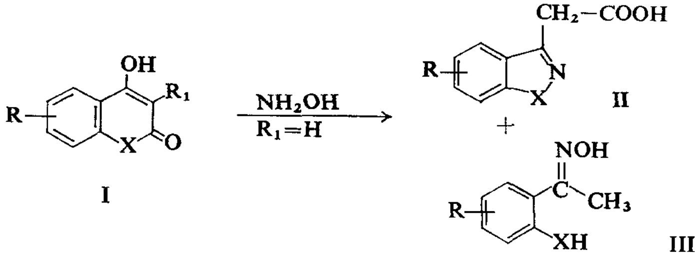

FIG. 1. SYNTHETIC ROUTE TO 1,2-BENZISOXAZOLE AND 1,2-BENZISOTHIAZOLE-3-ACETIC ACIDS.

When X = O, the acids II can be obtained with yields ranging from 50 to 90 per cent, together with a small amount of the corresponding 2-hydroxyacetophenone oximes (III). 3-Substituted 4-hydroxycoumarins do not undergo any reaction, possibly due to steric hindrance, while the presence of a hydrogen bond could account for the lack of reactivity of 4,5-dihydroxycoumarin. In fact, 5-methoxy-4-hydroxycoumarin does actually react with good yields.

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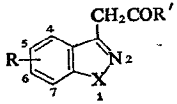
TABLE 1. 1,2-BENZISOXAZOLE- AND 1,2-BENZISOTHIAZOLE-3-ACETIC ACIDS AND THEIR DERIVATIVES*



No.	X	R	R′	Yields† %	M.p.° or b.p.°/Hg mm	Recrys. solvent‡	Formula	Analyses C%		H%		N%		S%	
								calc.	found	calc.	found	calc.	found	calc.	found
IV	O	$7CH_3$	OH	78	148–150 dec.	A	$C_{10}H_9NO_3$	62·82	63·19	4·75	5·02	7·33	7·05		
V	O	$7CH_3$	OCH_3	—	56–57	B	$C_{11}H_{11}NO_3$	64·38	64·60	5·40	5·20	6 83	6·92		
VI	O	$7CH_3$	OC_2H_5	—	110–114/0·2	—	$C_{12}H_{13}NO_3$	65·74	65·52	5·98	6·07	6·39	6·10		
VII	O	$4OCH_3$	OH	82	131–133 dec.	A	$C_{10}H_9NO_4$	57·97	58·10	4·38	4·60	6·76	6·79		
VIII	O	$4OCH_3$	OCH_3	—	78–80	C/B	$C_{11}H_{11}NO_4$	59·72	59·55	5·01	5·30	6·33	6·07		
IX	O	$4OCH_3$	OC_2H_5	—	60–61 135–138/0·7	B	$C_{12}H_{13}NO_4$	61·27	60·97	5·57	5·82	5·96	5·69		
X	O	$5OCH_3$	OH	80	146–149	A	$C_{10}H_9NO_4$	57·97	58 02	4·38	4·08	6·76	6·65		
XI	O	$5OCH_3$	OCH_3	—	70–71	B	$C_{11}H_{11}NO_4$	59·72	59·62	5·01	4·94	6·33	6·06		
XII	O	$5OCH_3$	OC_2H_5	—	140–145/2	—	$C_{12}H_{13}NO_4$	61·27	61·07	5·57	5·27	5·96	6·10		
XIII	O	$6OCH_3$	OH	75	174–175	A	$C_{10}H_9NO_4$	57·97	58·17	4·38	4·47	6·76	6·78		
XIV	O	$6OCH_3$	OCH_3	—	68–69	B	$C_{11}H_{11}NO_4$	59·72	60·01	5·01	5·28	6·33	6·60		
XV	O	$6OCH_3$	OC_2H_5	—	50–51	B	$C_{12}H_{13}NO_4$	61·27	61·20	5·57	5·50	5·96	6·17		
XVI	O	5OH	OH	69	170–172	A	$C_9H_7NO_4$	55·96	55·90	3·65	3·45	7·25	7·25		
XVII	O	5OH	OCH_3	—	105–107	D	$C_{10}H_9NO_4$	57·97	58·20	4·38	4·58	6·76	6·45		
XVIII	O	5OH	OC_2H_5	—	89–91	D	$C_{11}H_{11}NO_4$	59·72	59·42	5·01	4·79	6·33	6·10		
XIX	O	6OH	OH	51	186–187 dec	A	$C_9H_7NO_4$	55·96	56·18	3·65	3·98	7·25	7·50		
XX	O	6OH	OCH_3	—	115–117	D	$C_{10}H_9NO_4$	57·97	58·20	4·38	4·63	6·76	6·57		
XXI	O	6OH	OC_2H_5	—	104–106	D	$C_{11}H_{11}NO_4$	59·72	60·00	5·01	5·30	6·33	6·44		
XXII	O	$6NO_2$	OH	50	139–141 dec.	A	$C_9H_6N_2O_5$	48·66	48·90	2·72	3·00	12·61	12·58		
XXIII	O	$6NO_2$	OCH_3	—	109–111	E	$C_{10}H_8N_2O_5$	50·85	50·95	3·41	3·26	11·86	11·92		

XXIV	O	6NO$_2$	OC$_2$H$_5$	—	52–53	D	$C_{11}H_{10}N_2O_5$	52·80	52·80	4·03	3·98	11·20	11·50		
XXV	O	5Br	OH	90	175–177 dec.	E/A	$C_9H_6BrNO_3$	42·21	42·27	2·36	2·58	5·47	5·48		
XXVI	O	5Br	OCH$_3$	—	83–85	B	$C_{10}H_8BrNO_3$	44·46	44·62	2·98	3·14	5·18	5·42		
XXVII	O	5Br	OC$_2$H$_5$	—	72–74	B	$C_{11}H_{10}BrNO_3$	46·50	46·79	3·55	3·29	4·93	5·05		
XXVIII	O	5I	OH	43	152–156 dec.	E/A	$C_9H_6INO_3$	35·67	35·85	1·99	2·22	4·62	4·37		
XXIX	S	H	OH	35	153–155 dec.	A	$C_9H_7NO_2S$	55·96	55·75	3·65	3·89	7·25	7·50	16·57	16·22
XXX	S	H	OCH$_3$	—	34–35 122/0·3	F	$C_{10}H_9NO_2S$	57·97	57·70	4·38	4·37	6·76	7·00		
XXXI	S	H	OC$_2$H$_5$	—	49–50 220–230/5	F	$C_{11}H_{11}NO_2S$	59·72	59·73	5·01	5·10	6·33	6·51	14·46	14·08
XXXII	S	H	NH$_2$	—	188–190 dec.	E	$C_9H_8N_2OS$	56·25	56·50	4·20	4·47	14·58	14·64	16·65	16·24
XXXIII	S	H	NHCH$_3$	—	133–134 dec.	A	$C_{10}H_{10}N_2OS$	58·25	58·51	4·89	4·85	13·58	13·90		
XXXIV	S	H	NHNH$_2$	—	198–199 dec.	E	$C_9H_9N_3OS$	52·17	52·28	4·38	4·55	20·28	19·96	15·44	15·18
XXXV	S	H	NHNHCOCH$_3$	—	238–239 dec.	A	$C_{11}H_{11}N_3O_2S$	53·01	52·97	4·45	4·52	16·86	16·90	12·84	12·49
XXXVI	S	5Cl	OH	50	152–153 dec.	A	$C_9H_6ClNO_2S$	47·49	47·45	2·66	2·40	6·15	6·37	14·08	13·68
XXXVII	S	5Cl	OCH$_3$	—	71–72	G/A	$C_{10}H_8ClNO_2S$	49·70	49·48	3·34	3·37	5·79	5·51	13·27	12·98
XXXVIII	S	5Cl	OC$_2$H$_5$	—	24–25 140–143/0·07	F	$C_{11}H_{10}ClNO_2S$	51·67	51·87	3·94	4·11	5·48	5·76		
XXXIX	S	5Cl	NH$_2$	—	174–175 dec.	A	$C_9H_7ClN_2OS$	47·69	47·93	3·11	3·31	12·36	12·11	14·14	13·89
XL	S	5Cl	NHNH$_2$	—	206–207 dec.	E	$C_9H_8ClN_3OS$	44·73	45·01	3·34	3·60	17·39	17·40	13·27	13·30
XLI	S	5NO$_2$	OH	30	149–151 dec.	A	$C_9H_6N_2O_4S$	45·39	45·60	2·54	2·68	11·76	11·53	13·44	13·15
XLII	S	5NO$_2$	OCH$_3$	—	87–88	E/A	$C_{10}H_8N_2O_4S$	47·62	47·46	3·20	3·49	11·11	11·10	12·69	12·87
XLIII	S	5NO$_2$	OC$_2$H$_5$	—	72–74	E	$C_{11}H_{10}N_2O_4S$	49·63	49·83	3·79	3·86	10·52	10·92	12·02	11·80
XLIV	S	5NO$_2$	NH$_2$	—	183–185 dec.	A	$C_9H_7N_3O_3S$	45·57	45·75	2·98	2·79	17·72	18·01	13·49	13·20
XLV	S	5NO$_2$	NHNH$_2$	—	194–196 dec.	E	$C_9H_8N_4O_3S$	42·86	42·98	3·20	3·28	22·22	22·42	12·69	12·91

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* I.r. and u.v. spectra of the compounds reported in the table are in agreement with the proposed structures.

† Almost quantitative when no figures are given.

‡ A = water; B = *n*-hexane; C = CH_2Cl_2; D = EtOAc–petrol; E = ethanol; F = petrol; G = acetone.

When X = S, the reaction, carried out on 4-hydroxy- and 6-chloro-4-hydroxy-1-thiocoumarin is analogous, even if the yields are lower. However, owing to the difficulties in synthesizing the starting material, 5-nitro-1,2-benzisothiazole-3-acetic acid was obtained by direct nitration of 1,2-benzisothiazole-3-acetic acid.

Besides the analogy with the reaction of 4-hydroxycoumarins, the structures of the products obtained from 4-hydroxy-1-thiocoumarins and hydroxylamine were established by means of their chemical reactions, such as the decarboxylation of XXIX to the already known 3-methyl-1,2-benzisothiazole,[2] and the easy formation of its esters, amides, and hydrazides with the classical methods. I.r., u.v. and NMR spectra were similar to that of the corresponding benzisoxazole derivatives and gave spectroscopic evidence for the proposed structures.

The results of the determination of auxin-like activity with the slit-pea-stem curvature test[3] are shown in Table 2. Only the data of the most active compounds are reported, and compared with the activity of indole-3-acetic acid, used as a standard. It appears from the table that 1,2-benzisothiazole-3-acetic acid is more active than the corresponding benzisoxazole compound and that the halogenation of the nucleus increases the activity in both products, in analogy with the results of Porter for indole-3-acetic acid.[4] Consequently, 5-chloro-1,2-benzisothiazole-3-acetic acid is a very active compound, with an activity about three times that of heteroauxin.

TABLE 2. RESULTS OF THE SLIT-PEA-STEM CURVATURE TEST

Compound	Minimum active concn (M/l.)	Auxin activity (% of IAA*)
IAA	$2{\cdot}5 \times 10^{-6}$	100
1,2-benzisoxazole-3-acetic acid†	$50{\cdot}0 \times 10^{-6}$	5
XXV	$50{\cdot}0 \times 10^{-6}$	5
XXVIII	$25{\cdot}0 \times 10^{-6}$	10
XXIX	$10{\cdot}0 \times 10^{-6}$	25
XXXVI	$0{\cdot}75 \times 10^{-6}$	330
XLI	$50{\cdot}0 \times 10^{-6}$	5

* IAA = indole-3-acetic acid.
† See Ref. 1.

EXPERIMENTAL*

Starting Materials

4-Hydroxycoumarins, all known, were synthesized by the Boyd method,[5] according to the procedure previously described[6] for 6-bromo-4-hydroxycoumarin.[7] In this manner 8-methyl-4-hydroxycoumarin,[8]

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* M.ps were determined in capillary tubes on a Büchi apparatus and are uncorrected. Chemical and physical characteristics of the reported compounds, as well as their analytical data, are indicated in Table 2.

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[8] R. ANSCHUTZ, *Ann. Chem.* **379**, 341 (1911).

5-methoxy-4-hydroxycoumarin,[9] 6-methoxy-4-hydroxycoumarin,[10] and 7-methoxy-4-hydroxycoumarin[11] were obtained. 4,7-Dihydroxycoumarin[11] and 4,6-dihydroxycoumarin[12] were obtained from the corresponding 7- and 6-methoxy-4-hydroxycoumarins with the procedure described below. 7-Nitro-4-hydroxycoumarin and 6-iodo-4-hydroxycoumarin were prepared according to the methods reported, respectively by Julia[13] and Covello.[14] 4-Hydroxy-1-thiocoumarin[15] and 6-chloro-4-hydroxy-1-thiocoumarin[16] were obtained with the method of Ziegler.[15]

4,6- *and* 4,7-*Dihydroxycoumarins*

A mixture of the appropriate methoxy-4-hydroxycoumarin (0·5 g) HI (10 ml, d = 1·7), Ac_2O (5 ml), and HOAc (5 ml) was refluxed for 30 min. After removal of the solvent under vacuum, ground ice was added to the residue and the white solid obtained crystallized from aq. EtOH. The yields are 80–85%. The properties of the two compounds are that reported by the literature.[11,12]

Substituted 1,2-*Benzisoxazole*-3-*acetic Acids and Derivatives* (*IV–XXVIII*)

The appropriate 4-hydroxycoumarin (0·1 M) was dissolved in a solution of free hydroxylamine obtained from Na (0·3 M) and NH_2OH. HCl (0·3 M) in ethanol (200 ml). The solution was refluxed 9 hr, the solvent evaporated, the residue added with an aqueous solution of $NaHCO_3$ and extracted with ether. From the ether solution about 10% of the corresponding 2-hydroxyacetophenone oxime was obtained. The alkaline solution, acidified with 2N HCl, gave the substituted 1,2-benzisoxazole-3-acetic acids. They were characterized through their methyl and ethyl esters obtained by boiling for a few hr in the corresponding alcohol with a small amount of H_2SO_4.

1,2-*Benzisothiazole*-3-*acetic and* 5-*Chloro*-1,2-*benzisothiazole*-3-*acetic Acids* (*XXIX, XXXVI*)

The appropriate 4-hydroxy-1-thiocoumarin (25 mM), was dissolved in a solution of NH_2OH (75 mM) in alcohol, obtained as described above. The solution was heated at 75–80° for 6 hr, then concentrated and separated between a saturated $NaHCO_3$ solution and ether. The alkaline solution, acidified with 2N HCl, gave the 1,2-benzisothiazole-3-acetic acids. They were characterized by their methyl and ethyl esters, obtained as described above. To confirm the structure of these compounds the NMR spectrum of the methyl ester (XXX) was recorded: it shows the following signals (CCl_4): $\delta = 3{\cdot}6$ ppm (s, CH_3); $\delta = 4{\cdot}05$ ppm (s, CH_2); $\delta = 7{\cdot}1$–$8{\cdot}0$ ppm (m, 4 aromatic protons).

5-*Nitro*-1,2-*benzisothiazole*-3-*acetic Acid* (*XLI*)

1,2-Benzisothiazole-3-acetic acid (0·5 g) was slowly added to a 1:1 mixture of conc. H_2SO_4 and conc. HNO_3, stirring and cooling with water; the product slowly dissolves. After 15 min the solution was poured into ground ice, and the yellow solid obtained filtered and thoroughly washed with water. 5-Nitro-3-methylbenzisothiazole[2] was obtained as a byproduct of this reaction, due to the easy decarboxylation of XLI in acidic medium.

5-*Nitro*-1,2-*benzisothiazole*-3-*acetic Acid, Methyl* (*XLII*) *and Ethyl Esters* (*XLIII*)

They were prepared in the same way as XLI, starting from the methyl and ethyl esters of 1,2-benzisothiazole-3-acetic acid.

The esters were saponified to XLI, by dissolving them in hot 1N NaOH, followed by immediate cooling and acidification of the solution with 2N HCl. Even following this procedure, 5-nitro-3-methylbenzisothiazole was invariably formed as a byproduct.

Substituted 1,2-*Benzisothiazole*-3-*acetamides* (*XXXII, XXXIX, XLIV*)

The methyl ester of the appropriate 1,2-benzisothiazole-3-acetic acid (0·5 g) was dissolved in the minimum amount of alcohol, and treated with an excess of conc. NH_4OH. After few hr at room temp., water was added to precipitate the amides.

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1,2-*Benzisothiazole*-3-*acetic Acid*, N-*Methyl Amide* (*XXXIII*)

1,2-Benzisothiazole-3-acetic acid methyl ester (XXX) (0·5 g) was dissolved in the minimum amount of MeOH and treated with an excess of $MeNH_2$ (0·5 ml of 35% H_2O solution). The solution was kept at room temp. for a few days, then concentrated and treated with water to obtain the product.

Hydrazides of Substituted 1,2-*Benzisothiazole*-3-*acetic Acids* (*XXXIV, XL, XLV*)

The methyl ester of the suitable 1,2-benzisothiazole-3-acetic acid were dissolved in the minimum amount of alcohol and added with an excess of N_2H_4. After a few hr at room temp., the hydrazide was obtained as a white precipitate.

N-*Acetyl*-N′-(1,2-*benzisothiazole*-3)-*acetyl-hydrazide* (*XXXV*)

1,2-Benzisothiazole-3-acetic hydrazide (XXXIV) (0·5 g) was dissolved at room temp. in 5 ml Ac_2O and left to stand overnight. After addition of water, the product was obtained as a white solid.

Decarboxylation of 1,2-*Benzisothiazole*-3-*acetic Acid*

1,2-Benzisothiazole-3-acetic acid (2·0 g) CuO (0·30 g) and quinoline (8·0 ml) were heated at 180–185° for 0·5 hr. The cooled mixture was treated with 2N HCl till the solution was slightly acidic, and extracted with ether. The ether solution was washed with $NaHCO_3$ and dried. After evaporation of the solvent a yellow oil was obtained that, distilled, had the same i.r. spectrum of a true sample of 3-methyl-1,2-benzisothiazole. With ethanolic HCl it gave an hydrochloride with the characteristics described in the literature.[2]

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